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ABSTRACT

This document is an instructional module package prepared in objective form for use by an instructor familiar with multiple tube and membrane filter techniques for determining fecal coliform concentrations in a wastewater sample. Included are objectives, instructor guides, student handouts and transparency masters. This module considers proper laboratory practices, proper sampling, equipment and media preparation, test procedures and data interpretation. (Author/RH)

FECAL COLIFORM DETERMINATIONS

Training Module 5.115.3.77

Prepared for the

Iowa Department of Environmental Quality
Wallace State Office Building
Des Moines, Iowa 50319

by

Kirkwood Community College
6301 Kirkwood Boulevard, S. W.
P. O. Box 2068
Cedar Rapids, Iowa 52406

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September, 1977

The mention of trade names, or use of manufacturers technical bulletins, diagrams depicting specific equipment, or the commercial product in this module is, for illustration purposes, and does not constitute endorsement or recommendation for use by Kirkwood Community College nor by the Iowa Department of Environmental Quality.

Module No:	Module Title: Fecal Coliform Determination in Wastewater and Wastewater Effluent
Approx. Time: 29½ hours	Submodule Title 1. Multiple Tube Technique 2. Membrane Filter Technique

Objectives:

Upon completion of this module, the participant should be able to determine the fecal coliform density in a given sample by the multiple tube and/or the membrane filter technique.

Instructional Aids:

Handout A
Handout B
Transparancies
Necessary laboratory equipment

Instructional Approach:

Discussion
Demonstration
Laboratory Practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Basic Laboratory Skills module.

Class Assignments:

Read handouts A & B
Test a given sample by the multiple tube method
Test a given sample by the membrane filter technique

Module No:	Topic: SUMMARY

Instructor Notes:	Instructor Outline:
1. Handout A Transparancies	1. Discuss and demonstrate the multiple tube method of fecal coliform determination.
2. Supply all necessary equipment for the participant to complete an actual fecal coliform determination by the multiple tube method. *	2. Have participant analyze a given sample for fecal coliform density by the multiple tube method.
3. Handout B Transparancies	3. Discuss and demonstrate the membrane filter method of fecal coliform determination.
4. Supply all necessary equipment for the participant to complete on actual fecal coliform determination by the membrane filter technique.*	4. Have participant analyze a given sample for fecal coliform density by the membrane filter technique.

The use of technical bulletins from the major manufacturers may be helpful in supplementing information in student handouts.

* See student handout for basic reagent and equipment list.

Module No:	Module Title: Fecal Coliform Determination in Wastewater and Wastewater Effluent
	Submodule Title: Multiple Tube Method
Approx. Time: 2 hrs.	Topic: Introduction

Objectives: Upon completion of this module, the participant should be able to:

1. Describe the need for monitoring fecal coliform bacteria in wastewater effluent.
2. Give the fecal coliform standards for wastewater effluent as set forth by the USEPA.

Instructional Aids:

Handout A - Section #1

Instructional Approach:

Discussion

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Basic Laboratory Skills module.

Class Assignments:

1. Read Handout A - Section 1
2. The learner will provide the number (Figure only) of samples required to be tested for their distribution system.

Module No:	Topic: Introduction

Instructor Notes:	Instructor Outline:
Handout A (Section 1)	1. Discuss the need for the determination for fecal coliform. 2. Discuss the number of samples to be tested as required by law for Fecal Coliform Determination. 3. Discuss the bacteriological standards/or sewage effluent required by USEPA. 4. Ask learner to determine the number of samples to be tested for his/her city or town.

Module No:	Module Title: Fecal Coliform Determination in Wastewater and Wastewater Effluent
	Submodule Title: Multiple Tube Method
Approx. Time: 1½ hrs.	Topic: Applicable Basic Laboratory Skills Review

Objectives: Upon completion of this module, the participant should be able to:

1. Describe necessity for laboratory practices including:
   a. Setting laboratory rules
   b. Proper glassware cleaning and storage
   c. Aseptic technique
2. Identify and properly use major laboratory equipment.
3. Explain proper sampling and sample dilution techniques.

Instructional Aids:

Handout A (Appendix A, B, & C)

Transparancies

Necessary laboratory reagents and equipment

Instructional Approach:

Lecture
Discussion
Laboratory Practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Basic Laboratory Skills module.

Class Assignments:

Read handout Appendix A, B, C

Practice using major laboratory equipment

Module No:	Topic: Applicable Basic Laboratory Skills Review

Instructor Notes:	Instructor Outline:
Review basic laboratory skills module.	
Handout A (Appendix A)	1. Discuss the importance of setting laboratory rules on: a. Clothing b. Safety and safety equipment c. Record keeping
Handout A (Appendix A)	2. Discuss and demonstrate proper methods of glassware cleaning, glassware storage and aseptic technique.
Handout A (Appendix A)	3. List and discuss the use of major laboratory equipment including: a. Autoclave b. Sterilizing oven c. Incubators d. Distillation unit e. Glassware washer f. Refrigerator
Handout A (Appendix B & C)	4. Discuss sampling and sample dilution
Put emphasis on areas of procedure where errors occur and their affect on outcome.	5. Have participants practice using major equipment.

Module No:	Module Title: Fecal Coliform Determination in Wastewater and Wastewater Effluent
	Submodule Title: Multiple Tube Method
Approx. Time: 3 hrs.	Topic: Equipment and Media Preparation

Objectives: Upon completion of this module, the participant should be able to:

1. List the bench equipment needed to plant and transfer water samples.
2. Describe and demonstrate proper preparation of:
   a. Culture media and culture tubes
   b. Sterile dilution water
   c. Equipment for test

Instructional Aids:

Transparancies
Handout A (Section #2)
Demonstration
Necessary laboratory reagents and equipment

Instructional Approach:

Discussion
Demonstration
Laboratory Practice

References:

Standard Methods for the Examination of Water and Wastewater, 14th Edition.

Basic Laboratory Skills module

Class Assignments:

Read handout A - Section #2
Prepare growth media
Prepare culture tubes

Module No:	Topic: Equipment and Media Preparation

Instructor Notes:	Instructor Outline:
Handout A (Section #2) Demonstration	1. List and demonstrate use of bench equipment needed to complete test procedure. 2. Discuss preparation, use, and storage of sterile dilution water. 3. Discuss and demonstrate preparation of: a. Culture media b. Culture tubes 4. Have learner practice preparation of culture media and tubes.

Module No:	Module Title: Fecal Coliform Determination in Wastewater and Wastewater Effluent
	Submodule Title: Multiple Tube Method
Approx. Time: 6 hours	Topic: Test Procedure

Objectives: Upon completion of this module, the participant should be able to:

1. List the characteristics of a positive test.
2. Discuss and demonstrate proper technique for:
   a. Planting sample
   b. Transferring growth from presumptive to confirmatory media
   c. Recording data obtained from analysis
3. Discuss importance of proper incubation times and temperatures.

Instructional Aids:

Handout A (Section #3)

Transparency

Necessary laboratory reagents and equipment

Instructional Approach:

Discussion
Demonstration
Laboratory Practice

References:

Standard Methods for the Examination of Water and Wastewater, 14th Edition.

Basic Laboratory Skills module

Class Assignments:
Read handout A - Section #3
Practice tube inoculations
1. Planting sample using pipet
2. Transferring growth using loop
Practice recording data

Module No:	Topic: Test Procedure

Instructor Notes:	Instructor Outline:
Handout A - Section #3 Demonstration	1. Discuss test procedure 2. Demonstrate: a. Use of pipet for planting sample. b. Use of loop for transferring growth 3. Describe appearance of a positive test result and what to do with it. 4. Demonstrate proper method of recording test data. 5. Discuss and demonstrate proper disposal of used culture tubes. 6. Have learner plant samples in 15 tubes and transfer the positive growth tubes and record data on worksheet.

Module No:	Module Title: Fecal Coliform Determination in Wastewater and Wastewater Effluent
	Submodule Title: Multiple Tube Method
Approx. Time: 2 hours	Topic: Data Interpretations

Objectives: Upon completion of this module, the participant should be able to:

1. Determine fecal coliform level found in sewage effluent sample tested.
2. Describe what to do if the results of the analysis are not within the normal range.
3. Given a set of test results determine whether the effluent is bacteriologically safe.

Instructional Aids:

Handout A - (Section #4)

Instructional Approach:

Discussion

References:

Standard Methods for the Examination of Water and Wastewater, 14th Edition.

Basic Laboratory Skills module

Class Assignments:

1. Read handout A - Section #4
2. Practice calculation and data interpretation

Module No:	Topic: Data Interpretations

Instructor Notes:	Instructor Outline:
Handout A (Section #4)	1. Discuss the acceptable Fecal Coliform level. 2. Discuss what to do if the results of the analysis are not within the Normal Range including: a. Increasing the frequency of testing b. Reporting to supervisor 3. Discuss factors which erroneously affect test results. a. Errors in sampling b. Errors in lab technique c. Errors in calculation 4. Discuss whether any given effluent is bacteriologically safe.

Module No:	Module Title: Fecal Coliform Determination in Wastewater and Wastewater Effluent
	Submodule Title: Membrane Filter Technique
Approx. Time: ½ hour	Topic: Introduction

Objectives: Upon completion of this module the participants should be able to:

1. Explain the importance of monitoring fecal coliforms in wastewater effluent and polluted water.

2. Describe:

   a. The fecal coliform group
   b. Water quality standards with respect to fecal coliforms in wastewater effluent and the receiving stream.

Instructional Aids:

Handout B (Section #1)

Transparencies

Instructional Approach:

Discussion

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.

2. Basic Laboratory Skills module.

Class Assignments:

Read handout B - Section #1

Module No:	Topic: Introduction

Instructor Notes:	Instructor Outline:
Handout B - Section #1 Transparancies	1. Discuss the relationship between fecal coliforms and pathogenic (disease causing) bacteria. 2. Describe the morphology of the fecal coliform bacteria including: a. Size b. Shape c. Colony morphology on m-FC agar 3. Discuss fecal coliform standards for wastewater effluent and receiving stream.

Module No:	Module Title: Fecal Coliform Determination in Wastewater and Wastewater Effluent
	Submodule Title: Membrane Filter Technique
Approx. Time: 1½ hours	Topic: Applicable Basic Skills Review

Objectives: Upon completion of this module the participants should be able to:

1. Describe the necessity for laboratory practices including:
   a. Setting laboratory rules
   b. Proper glassware cleaning and storage
   c. Aseptic technique
2. Identify and properly use major laboratory equipment.
3. Explain proper sampling and sample dilution techniques.

Instructional Aids:

Handout B (Appendixes A, B, & C)

Transparancies

Necessary laboratory reagents and equipment

Instructional Approach:

Lecture
Discussion
Laboratory Practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Basic Laboratory Skills module.

Class Assignments:

1. Read handout B Appendixes A, B, & C.
2. Practice using major laboratory equipment.

Module No:	Topic: Applicable Basic Skills Review
Instructor Notes:	**Instructor Outline:**
Review Basic Laboratory Skills Module	
Handout B - Appendix A	1. Discuss the importance of setting laboratory rules on: a. Clothing b. Safety and safety equipment c. Record keeping
	2. Discuss and demonstrate proper methods of glassware cleaning, glassware storage, and aseptic technique.
	3. List and discuss the use of major laboratory equipment including: a. Autoclave b. Sterilizing oven c. Incubators d. Distillation unit e. Glassware washer f. Refrigerator
Handout B - Appendixes B & C	4. Discuss sampling and sample dilution.
Put emphasis on areas of procedure where errors occur and their affect on outcome.	5. Have participants practice using major equipment.

Module No:	Module Title: Fecal Coliform Determination in Wastewater and Wastewater Effluent
	Submodule Title: Membrane Filter Technique
Approx. Time: 3 hours	Topic: Equipment and Media Preparation

Objectives: Upon completion of this module the participant should be able to:

1. List the bench equipment and expendables needed to filter the water sample culture the membrane and count the colonies.

2. Describe and demonstrate proper preparation of:

   a. Culture media
   b. Sterile dilution water
   c. Equipment and expendables for test

Instructional Aids:

Handout B - Section #2
Transparancies
Demonstration
Necessary laboratory reagents and equipment

Instructional Approach:

Discussion
Demonstration
Laboratory Practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.

2. Basic Laboratory Skills module.

Class Assignments:

1. Read handout B Section #2.
2. Practice preparing:
   a. Culture media
   b. Sterile dilution water
3. Practice preparing bench equipment and expendables.

Module No:	Topic: Equipment and Media Preparation
Instructor Notes:	Instructor Outline:
Handout B - Section #2 Transparancies	1. List and demonstrate use of bench equipment and expendables needed to complete test procedure. 2. Discuss preparation, use, and storage of sterile dilution water. 3. Discuss and demonstrate preparation of culture media. 4. Have participant practice: a. Preparing culture media b. Preparing dilution water c. Wrapping bench equipment for sterilizing.

Module No:	Module Title: Fecal Coliform Determination in Wastewater and Wastewater Effluent
	Submodule Title: Membrane Filter Technique
Approx. Time: 4 hours	Topic: Membrane Filtration Procedure

Objectives: Upon completion of this module the participant should be able to discuss and/or demonstrate proper technique for:

1. Dispensing media (both broth and agar)
2. Assembling filtration equipment
3. Filtering any volume of sample size
4. Plating and incubating inoculated membrane filter

Instructional Aids:

Handout B - Section #3
Transparancies
Demonstration
Necessary laboratory reagents and equipment

Instructional Approach:

Demonstration
Laboratory Practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Basic Laboratory Skills module.

Class Assignments:

Read Handout B - Section #3
Practice procedure by assembling equipment, filtering several dilutions of a water sample, and plating and incubating the cultured membrane filter.

Module No:	Topic: Membrane Filtration Procedure
Instructor Notes	Instructor Outline:
Handout B - Section #3 Demonstration	Discuss and demonstrate preparation of work area. 1. Disinfection 2. Equipment assembly 3. Dispensing M-FC agar and broth
Handout - Section #3 Demonstration and transparancy	Discuss and demonstration sample filtration 1. Placing membrane in funnel 2. Adding sample 3. Filtering and rinsing 4. Removal of filter from funnel
Handout B - Section #3 Demonstration and transparancy	Discuss and demonstrate culturing of membrane 1. Placing membrane on growth media 2. Incubation Have students practice all of the above.

Module No:	Module Title: Fecal Coliform Determination in Wastewater and Wastewater Effluent
	Submodule Title: Membrane Filter Technique
Approx. Time: 2 hours	Topic: Counting Procedure

Objectives: Upon completion of this module the participant should be able to:

1. Determine by examination, which membrane each sample set requires counting.
2. Describe proper counting methodology.
3. Demonstrate ability to differentiate between fecal coliform and non-fecal coliform colonies and count fecal coliform colonies accurately.

Instructional Aids:

Handout B - Section #4
Transparancy
Demonstration
Necessary laboratory reagents and equipment

Instructional Approach:

Discussion
Demonstration
Laboratory Practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Basic Laboratory Skills module.

Class Assignments:
Read Handout B - Section #4
Practice counting fecal coliform colonies on membrane filters.

Module No:	Topic: Counting Procedure
Instructor Notes:	Instructor Outline:
Handout B - Section #4 Transparancies Demonstration	Discuss and demonstrate how to choose correct membrane to count and proper counting methodology. Discuss colony differentiation including: a. Colony color b. Colony shape c. Colony size Have students practice counting colonies on membrane filters.

Module No:	Module Title: Fecal Coliform Determination in Wastewater and Wastewater Effluent
	Submodule Title: Membrane Filter Technique
Approx. Time: 2 hrs.	Topic: Data Interpretation and Evaluation

Objectives: Upon completion of this module the participant should be able to:

1. Compute number of fecal coliforms per 100 ml. given dilution mls. filtered, and fecal coliform count.
2. Determine whether sample meets standards given water source, table of acceptable limits, and fecal coliform count.
3. Identify necessary action given a result not meeting standards.

Instructional Aids:

Handout B - Section #5
Transparancy
Demonstration

Instructional Approach:

Discussion B - Section #5
Demonstration
Practice Problem

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Basic Laboratory Skills module.

Class Assignments:

Read handout B - Section #5
Calculate # of fecal coliforms per 100 ml. sample from membrane counted.
Answer sample problem question.

Module No:	Topic: Data Interpretation and Evaluation
Instructor Notes:	Instructor Outline:
Handout B - Section #5 Transparancies	Demonstrate calculating # fecal coliforms per 100 mls. Have students do practice problem. Have students calculate # fecal coliforms per 100 mls. for sample they filtered. Discuss whether sample meets standards and what to do if it does not.

HANDOUT A

MULTIPLE TUBE TECHNIQUE FOR THE DETERMINATION OF FECAL COLIFORMS IN WASTEWATER & WASTEWATER EFFLUENT

SECTION 1: INTRODUCTION TO FECAL COLIFORM TESTING

I. FECAL COLIFORMS ARE A GROUP OF BACTERIA

A. Need oxygen to survive

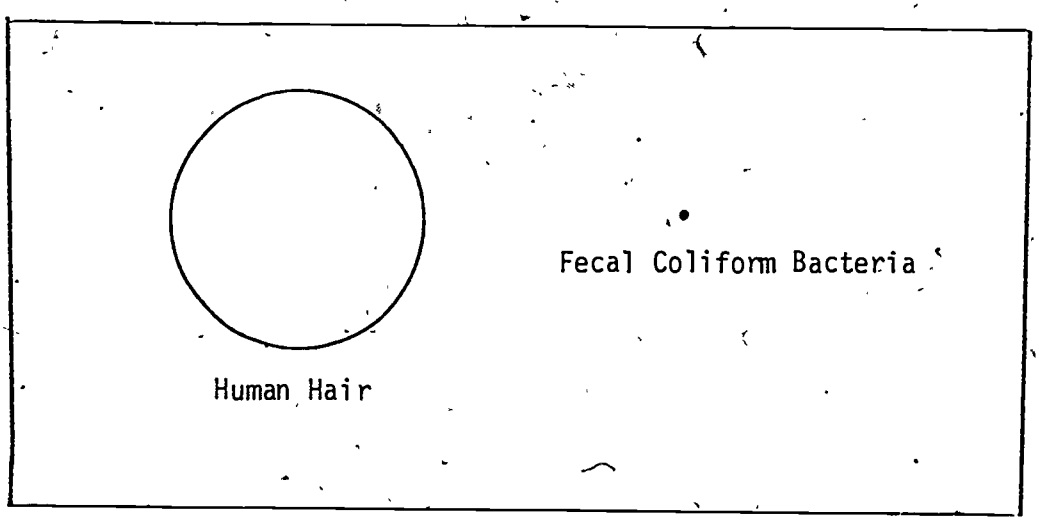
B. Rod shaped

C. Gram negative

D. Ferment the sugar lactose with gas production within 24 hrs. at 44.5° C.

E. Found in fecal matter only

F. See Figure #1 for size comparison



II. LARGE NUMBERS OF FECAL COLIFORMS IN WASTEWATER EFFLUENT MAY INDICATE

A. Fecal matter present, therefore disease causing organisms present.

B. Insufficient chlorination, therefore disease causing organisms still alive also.

III. WATER QUALITY STANDARDS FOR WASTEWATER EFFLUENT AND DISCHARGE WATERS

A. River or discharge water has maximum allowable fecal coliform level of 200/100 mls.

B. Sewage effluent has maximum allowable fecal coliform level of 400/100 mls.

SECTION 2: BENCH EQUIPMENT AND MEDIA PREPARATION

I. LIST OF BENCH EQUIPMENT, MEDIA AND REAGENTS

A. Bench equipment

1. Hot plate
2. Balance with 0.5 gm. sensitivity
3. pH meter
4. Bunsen type burner
5. Pipet soaking jar

B. Glassware

1. 1 l. erlynmeyer flasks
2. Sample bottles
3. Graduated cylinders
4. 100 ml. dilution blanks
5. Test tubes - 150 x 18 mm - borosilicate glass plus caps
6. Test tubes - 75 x 10 mm - borosilicate glass
7. Pipets - 10 ml and 1 ml - calibrated in 0.1 ml. - T.D. or Mohr
   a. Sterile, disposable, cotton plugged, individually wrapped - or -
   b. Borosilicate glass with aluminum or steel can for sterilizing in.

C. Expendables

1. Non-absorbant cotton
2. Brown Kraft wrapping paper
3. Aluminum foil
4. Rubber gloves
5. Paper towels
6. Sponge
7. Marking pens

D. Safety Equipment

1. Fire extinguisher
2. Fire blanket
3. First aid kit
4. Emergency shower
5. Emergency eye wash

E. Reagents and Media

1. Disinfectant
2. Peptone or $KH_2 PO_4$
3. 1 N NaOH
4. 1 N HCl
5. Lactose broth or Lauryl tryptose sulfate broth
6. EC broth
7. Distilled water

II. BENCH EQUIPMENT PREPARATION & FUNCTION

A. Hot plate

1. Keep top clean for even heat
2. Used to heat solutions to aid in dissolution

B. Balance with 0.5 gm sensitivity

1. Keep clean and checked for accuracy.
2. Used to weigh dry media and reagents

C. pH meter

1. Check for accuracy with known standards
2. Used for checking pH of prepared media

D. Bunsen type burner

1. Clean gas jet to prevent clogging

2. Adjust for a blue flame with good cone

3. Used for sterilization of inoculating loop

E. Pipet soaking jar

1. Clean weekly to remove old pipets and spent disinfection

2. Holds used pipets until cleaned or disposed of

III. GLASSWARE PREPARATION & FUNCTION

A. Function of each item listed

1. 1 l. erlynmeyer flasks used for media preparation must be washed and dried.

2. Graduated cylinders used for measuring liquid volumes must be washed and dried.

3. 100 ml. dilution blanks

a. Washed & dried

b. Filled with 99 ml. sterile distilled buffered water

c. Sterilized

d. Used to dilute samples if necessary

4. 18 x 150 mm test tubes + caps

a. Washed & dried

b. Filled with growth media

c. Capped and sterilized

d. Used to grow bacteria

5. 10 x 77 mm test tubes

a. Inverted inside, 18 x 150 mm filled test tubes prior to sterilization

b. Used to trap gas produced by bacterial growth

6. Pipets used for measuring sample

   a. Sterile disposable pipets need no preparation but must be stored in a clean dry place.

   b. Borosilicate glass must be washed, dried, plugged and sterilized in proper container.

IV. REAGENT AND MEDIA PREPARATION AND FUNCTION

A. Use distilled water only

B. Sterile distilled buffered water - 2 types

1. Phosphate buffered water

   a. Stock solution

      1. Dissolve 34 gms. KH_2PO_4 in 500 mls. distilled water in a volumetric flask.

      2. Adjust to pH 7.2 with 1 N NaOH

      3. Dilute to 1 l. with distilled water

   b. To make buffered water for sample dilution

      1. Add 1.25 mls stock to 1 l. distilled water

      2. Mix, dispense and sterilize

2. Peptone dilution water

   a. Stock solution

      1. Dissolve 10 gms. peptone in 100 mls. water

      2. To store sterilze 15 min. at 121° C. in an autoclave and store in refrigerator.

      3. Discard if it becomes cloudy

   b. To make dilution water

      1. Add 10 ml. stock to 1 l. distilled water

      2. Mix, dispense and sterilize

3. Sterilization of buffered and dilution water

   a. Dispense 99 mls. plus 4 mls. (to allow for evaporation) in 100 ml. dilution blanks.

b. Sterilize in an autoclave for 20 min. at 121° C. (15 psi)

c. Use slow exhaust

d. Sterilize with caps loose

e. Tighten caps when removed from autoclave

C. Sodium Thiosulfate Solution

1. Stock solution

a. Weigh 10 gms. of Sodium thiosulfate

b. Dissolve in 50 - 60 mls. distilled water in a 100 ml. volumetric flask

c. Add distilled water to bring to a final volume of 100 mls.

d. Transfer to a stoppered, 100 ml. labeled bottle and store in refrigerator

2. For use as a dechlorinating agent

a. Transfer 0.1 ml (for each 40 oz. capacity) to sample bottle with 1 ml. pipet

D. Lauryl tryptose sulfate broth (LTSB) for presumptive test

1. Order in amounts to fit needs

a. 1 lb. bottle will make enough media for 120 samples

b. Available in 1/4 lb. amounts

2. Keep bottle tightly closed

a. Dehydrated media is hygroscopic

b. Caked media must be discarded

3. Prepare according to manufacturer's instructions

a. In strengths appropriate for sample volumes used

b. In amounts applicable to use

c. Adjust pH if necessary

4. Dispense 10 ml. ± 0.5 ml into each clean, dry 150 x 18 mm test tube

5. Insert 1 clean, dry 75 x 10 mm test tube open end down into larger tube

6. Cap large tube
7. Label as to strength
8. Sterilize
   a. Within 1 hr. of preparation
   b. Cycle of 15 min. at 121° C. in an autoclave set for slow exhaust
   c. Remove from autoclave immediately upon completion of cycle
9. Cool to room temperature and check pH
   a. pH = 6.8 - 7.0
   b. Discard if not within limits
10. Store in cool place for not more than 1 month

E. EC broth

1. Order in amounts to fit needs
   a. 1 lb. bottle will make enough media for 1,250 confirmations
   b. Available in 1/4 lb. amounts
2. Keep bottle tightly closed
   a. Dehydrated media is hygroscopic
   b. Caked media must be discarded
3. Prepare
   a. According to manufacturer's instructions
   b. In amounts applicable to use
   c. Adjust pH if necessary
4. Dispense 10 ml. ± 0.5 ml. into each clean, dry 150 x 18 mm test tube
5. Insert 1 clean, dry 75 x 10 mm test tube open end down into larger tube.
6. Cap large tube
7. Label as to strength

8. Cool to room temperatur and check pH

   a. pH = 6.9

   b. Discard if not 6.9

9. Store in cool place for not more than 1 month

SECTION 3: MULTIPLE TUBE PROCEDURE

I. DATA SHEET PREPARATION

II. WORK AREA PREPARATION

A. Wash hands and disinfect work bench top

1. Lowers possibility of sample contamination leading to duplication of work.

B. Assemble and label culture tubes

1. Place 5 tubes of the appropriate strength Lauryl tryptose sulfate broth for each dilution of each sample to be tested.

a. Use double strength for 10 ml. sample volume

b. Use single strength for 1 and 0.1 ml. sample volumes

2. Label tubes

a. Sample #

b. Sample volume inoculated

c. Position of tube in series of five

III. SAMPLE INOCULATION, INCUBATION ETC.

A. Inoculate tubes

1. Shake sample vigorously

2. For each sample

a. Deliver the 5 10-ml. sample volumes

b. Deliver the 5 1-ml. sample volumes

c. Deliver the 5 0.1-ml. sample volumes

3. Use sterile 10 ml. and 1 ml. pipets respectively

4. Use aseptic technique

B. Swirl tubes gently to mix

C. For poor quality effluent and untreated wastewater smaller decimal dilutions of 10 are used.

1. Counts expected to be greater than 2,400/100 mls.

2. Use media prepared accordingly

3. Use sterile 99 ml. dilution blanks for sample dilution

D. Incubate 24 ± 2 hrs. at 35 ± 0.5° C.

1. At end of 24 hrs. check for gas production

a. No gas - incubate additional 24 hrs. at 35 ± 0.5° C.

b. Positive gas production

1. Record on data sheet as positive

2. Confirm test results by

a. Transferring loopful to E. C. broth

1. Use aseptic technique

2. Use 3 mm loop

b. Labeling inoculated EC tube to correspond to positive LTSB tube

c. Incubating inoculated EC tube for 24 ± hrs. at 44.5 ± 0.2° C.

E. Re-incubated LTSB tubes

1. No gas production at end of incubation period

a. No further action

b. Record as negative on data sheet

2. Positive gas production

a. Record on data sheet as positive

b. Confirm test results by

1. Transferring loopful to EC broth

a. Use aseptic technique

b. Use 3 mm loop

2. Labeling inoculated EC tube to correspond to positive LTSB tube.

3. Incubating inoculated EC tube for 24 ± 2 hrs. at 44.5 ± 0.2° C.

F. After incubation period is completed for the inoculated EC tubes, check for gas production .

1. No gas production

a. No further action

b. Record as negative on data sheet

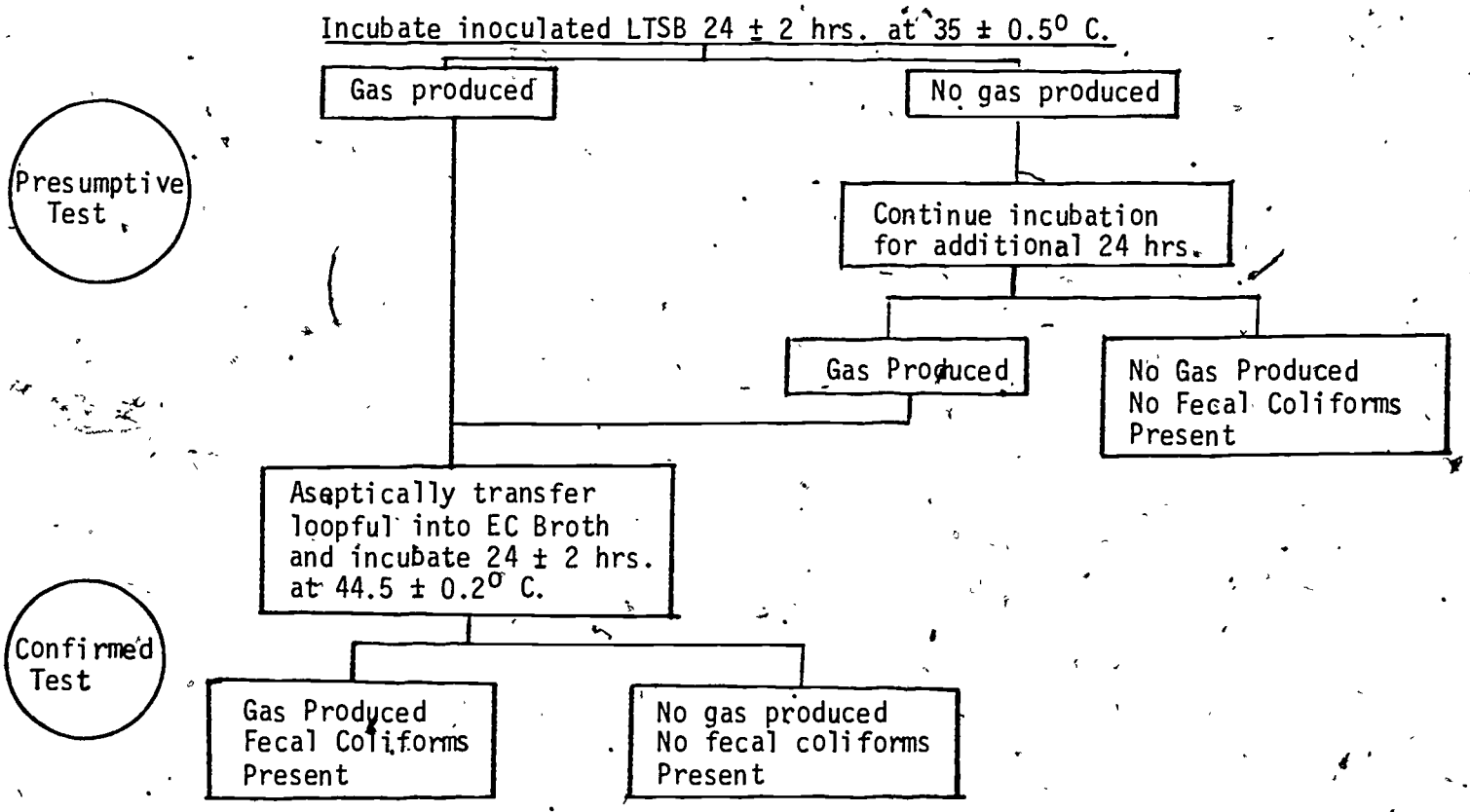
2. Gas production

a. Record on data sheet as positive

b. Indicates fecal coliform bacteria present

c. May be further confirmed by methods described in "Standard Methods for the examination of Water and Wastewater

II. SAMPLE INOCULATION & INCUBATION - SCHEMATIC



SECTION 4: PROCESSING USED GLASSWARE

I. CONTAMINATED BUT UNCULTURED GLASSWARE

A. Sterilization unnecessary

B. Empty contents down drain

C. Wash, rinse, dry as previously described

D. Prepare for next testing series

1. Prepare

2. Wrap or package

3. Sterilize

II. GLASSWARE CONTAINING CULTURES

A. Sterilize in an autoclave

B. Empty contents down drain

C. Wash, rinse and dry as previously described

D. Prepare for next testing series

1. Prepare

2. Wrap, package, cap etc.

3. Sterilize

III. DISPOSABLES

A. Discard in polypropylene bag

B. Sterilize in autoclave

C. Dispose of in garbage

SECTION 5: DATA INTERPRETATION & EVALUATION

I. RECORD THE NUMBER OF GAS POSITIVE TUBES FROM THE CONFIRMED TEST FOR EACH DILUTION OF EACH SAMPLE

Example

Sample A

Amt. inoculated	# positive
10 ml	5
1 ml	2
0.1 ml	1

II. USE CHART IN "STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER" TO DETERMINE THE "MPN INDEX" PER 100 MLS. (NUMBER OF FECAL COLIFORM BACTERIA PER 100 MLS.)

III. THE WASTEWATER EFFLUENT WILL BE CONSIDERED UNSAFE IF THE "MPN INDEX" EXCEEDS 400 FECAL COLIFORM BACTERIA PER 100 MLS. OF EFFLUENT

APPENDIX A - LABORATORY PREPARATION

I. SETTING LABORATORY RULES

A. Dress Code

1. Must wear lab coat or apron at all times
2. Shoes must have full foot protection
3. Long hair must be tied back
4. Must wear protective clothing where applicable
   a. Goggles or safety glasses
   b. Asbestos gloves

B. Safety Equipment

1. General Equipment
   a. Fire extinguisher
   b. Fire blanket
   c. First aid kit
   d. Emergency shower
   e. Emergency eye wash
2. Personal equipment for each employee
   a. Lab coat or apron
   b. Goggles
   c. Asbestos gloves
3. Safety rules
   a. Must be set and enforced by supervisor
   b. All accidents must be reported to supervisor

C. Record Keeping

1. Must be maintained at all times
2. Should include all:

a. Purchase records

b. Equipment specifications, warranties, maintenance and instruction manuals.

c. Accidnet reports

d. Testing data

e. Pertinent communications

f. Employee records

II. LABORATORY CLEANLINESS

A. Types of disinfectants

1. 70% Ethanol

2. Phenols i.e. O-Syl

3. Quaterniary ammonium compounds

4. Halogen compounds

5. Activated sialdehyde i.e. cidex

B. Use of disinfectants

1. Weekly

a. Wipe down all shelves removing all glassware and books

b. Wipe down all incubators, inside and outside

c. Wipe out inside of autoclave.

2. Daily

a. Wipe down tops of all counters, large pieces of equipment

3. Immediately before testing disinfect work area

4. Immediately disinfect spills

C. Sources of Contamination

1. Dirt around lab

2. Spilled samples or cultures

3. Un-autoclaved bacterial garbage

4. Chemical contamination from use of glassware for both Chemistry testing and Bacterial testing.

III. GLASSWARE WASHING

A. All glassware must be thoroughly washed in non-toxic detergent

1. i.e. Alconox

2. Removes bacterial scum from glassware

B. Rinse 6 - 12 times in hot tap water

1. Removes detergent residue

2. Residue is harmful to bacteria

C. Final rinse 1 - 3 times in distilled water

1. Removes mineral residue from tap water

2. Prevents water spotting

D. Air Dry

1. Any spot indicates dirt

2. Rewash before using

IV. PACKAGING EQUIPMENT AND STERILIZATION

A. Reasons for packaging

1. Creates a bacteria barrier

2. Allows for storage of sterile equipment

B. Proper labeling

1. Define contents

2. Date to aid in equipment rotation

C. Sterilization of equipment - 2 Acceptable Methods

1. Autoclave

a. All rubber, metal and glassware and some plastics

b. Normal cycle 15 min. 15 121° C.

c. Exhaust rapidly

2. Hot air sterilizing oven

   a. Dry glassware and metal objects only

   b. Normal cycle 1 hr. at 170° C.

   c. Allow to cool before use

   d. Package pipets in metal containers

   e. Package other equipment with aluminum foil

V. MAJOR LABORATORY EQUIPMENT

A. Autoclave

1. Before using read and follow manufacturers installation use and maintenance instructions and safety precautions.

2. Normal sterilization = 15 psi yielding 121° C. for 15 min.

3. Use to sterilize liquids and non-heat sensitive equipment

   a. Most plastics are not autoclavable and sterilized by manufacturer.

   b. Sterilized media and reagents must be removed from autoclave as soon as possible after autoclave is opened.

   c. Glassware may be sterilized in autoclave but must be allowed to dry before removing from autoclave.

B. Hot air Sterilizing Oven

1. Before using read and follow manufacturers installation, use, and maintenance instructions and safety precautions.

2. Normal Sterilization = 1 hour at 180° C.

3. Use to sterilize glass and metal only

   a. Rubber and plastics will melt.

   b. Liquids will evaporate and grow media components will be destroyed

C. 35° Incubator

1. Before using read and follow manufacturers installation and maintenance instructions and safety precautions.

2. Place in permanent location

   a. Out of drafts and direct sunlight

   b. Convenient to laboratory bench and electrical outlet

3. Install thermometer

   a. NBS (National Bureau of Standards) certified thermometer

   b. Mercury bulb of thermometer should be suspended in bottle filled with water.

   c. Locate centrally in incubator

4. Install shallow pan of water in bottom of incubator

   a. Maintains condition of saturated relative humidity required in bacteriological incubator.

   b. Check daily and fill as necessary to keep water in pan at all times.

5. Adjust temp. to $35^{o} \pm 0.5^{o}$ C.

   a. Follow manufacturers instructions

   b. Allow 1 hr. between temperature adjustments

   c. Record temp. of incubator daily

D. Water Distillation and Deionizing Unit

1. Before using, read and follow manufacturers installation, use and maintenance instructions and safety precautions.

2. Produces reagent grade water for use in making reagents and media and rinsing glassware.

E. Refrigerator

1. Set to maintain a 4^{o} C. temperature

2. Use to hold samples waiting to be tested and to store some prepared media and reagents.

F. Glassware washer

1. Before using, read and follow manufacturers installation, use and maintenance instructions and safety precautions.

2. Automatically washes and rinses glassware.

3. Do not use home dishwasher as it does not have proper plumbing.

APPENDIX B - COLLECTING SAMPLES FOR BACTERIOLOGICAL EXAMINATION

I. EQUIPMENT PREPARATION

A. Sample bottles must be:

1. At least 100 ml. capacity with a large neck opening.
2. Thoroughly cleaned with detergent, rinsed 6 times in hot tap water, rinsed finally in distilled-deionized water, then air dried.
3. Free from spots, scum, chips, cracks, excessive scratches and other damage on which bacteria may lodge.
4. Closed with preferably an all glass ground cap closure (but screw caps can be used providing liners are free from contamination and provide a non-leaking seal.
5. Sterilized in an autoclave at 121° C. for 15 min. with Kraft paper or tin foil hood covering caps and necks of bottles and slip of paper between bottleneck and glass stopper to prevent glass stopper from sticking.

B. Bottles intended for use in collection of chlorinated samples must have a 10% sodium thiosulfate solution added at the rate of 0.1 ml. for each 4 oz. bottle prior to sterilization and sterilized in bottle.

C. Labels must be:

1. Clean and unused
2. Attached to bottle by a means not affected by water (i.e. string or wire.)

D. Label markers must be:

1. Permanent type not affected by water
2. Able to mark on label

E. Sampling devices must be in working condition and properly maintained.

F. Germicide must be available to clean up spills but must not come in contact with sample or any equipment touched by sample.

G. Rubber gloves must fit and not be punctured.

H. Ice chest for transporting sample must be:

1. Sufficient size to accommodate all samples
2. Undamaged with tight cover so cold temperature can be maintained inside.

3. Filled with enough ice to quickly chill sample but little or no free water.

I. Refrigerator must be set at 2 - 10° C. and used if samples are not examined upon immediate return to lab.

II. SAMPLE COLLECTION

A. Minimum number of samples to be taken is based on flow and industry on line.

B. To take sample from spigot or tap:

1. Find spigot with direct main connection

2. Put on rubber gloves

3. Flush spigot at full flow for 2 - 3 min. to clear service line

4. If right handed, hold sample bottle near bottom with right hand and remove closure and paper hood with left hand (reverse if left handed). DO NOT LAY CLOSURE DOWN. Hold in such a way to protect closure and bottle from contamination.

5. Allow slip of paper between closure and bottle neck to fall to floor.

6. Thrust bottle into flowing water and allow bottle to fill about 3/4ths full. DO NOT RINSE, especially if bottle contains sodium thiosulfate to neutralize chlorine in sample.

7. Carefully replace closure and hood and secure.

8. Label bottle and place on ice in ice chest for transportation to laboratory.

C. To sample river, stream, [redacted]ke, etc.

1. Put on rubber gloves.

2. If right handed, hold sample bottle near bottom with right hand and remove closure and paper hood with left hand (reverse if left handed). DO NOT LAY CLOSURE DOWN. Hold in such a way to protect closure and bottle from contamination.

3. Allow paper strip between and bottle to fall to ground.

4. To fill sample bottle

a. Turn bottle neck opening down and plunge below surface of water quickly to prevent dechlorinating agent from running out.

b. Turn upward to face bottle opening into current to avoid contamination of water flowing into bottle with samplers hand.

c. Allow to fill to about 3/4 full. DO NOT OVERFILL especially if bottle contains a dechlorinating agent.

d. Lift quickly out of water and replace closure and hood.

5. Label bottle and place on ice in ice chest for transportation to laboratory.

III. COMMON ERRORS AND AFFECT ON RESULTS

A. No dechlorinating agent in bottle. Chlorine activity continues until sample tested so bacteria continue to die and coliform determination gives count which is lower than actual.

B. Sample not chilled when taken. Bacteria continue to multiply, so coliform determination gives count which is higher than actual.

C. Bottle or closure contaminated. Extra bacteria introduced, so coliform determination may give count which is higher than actual.

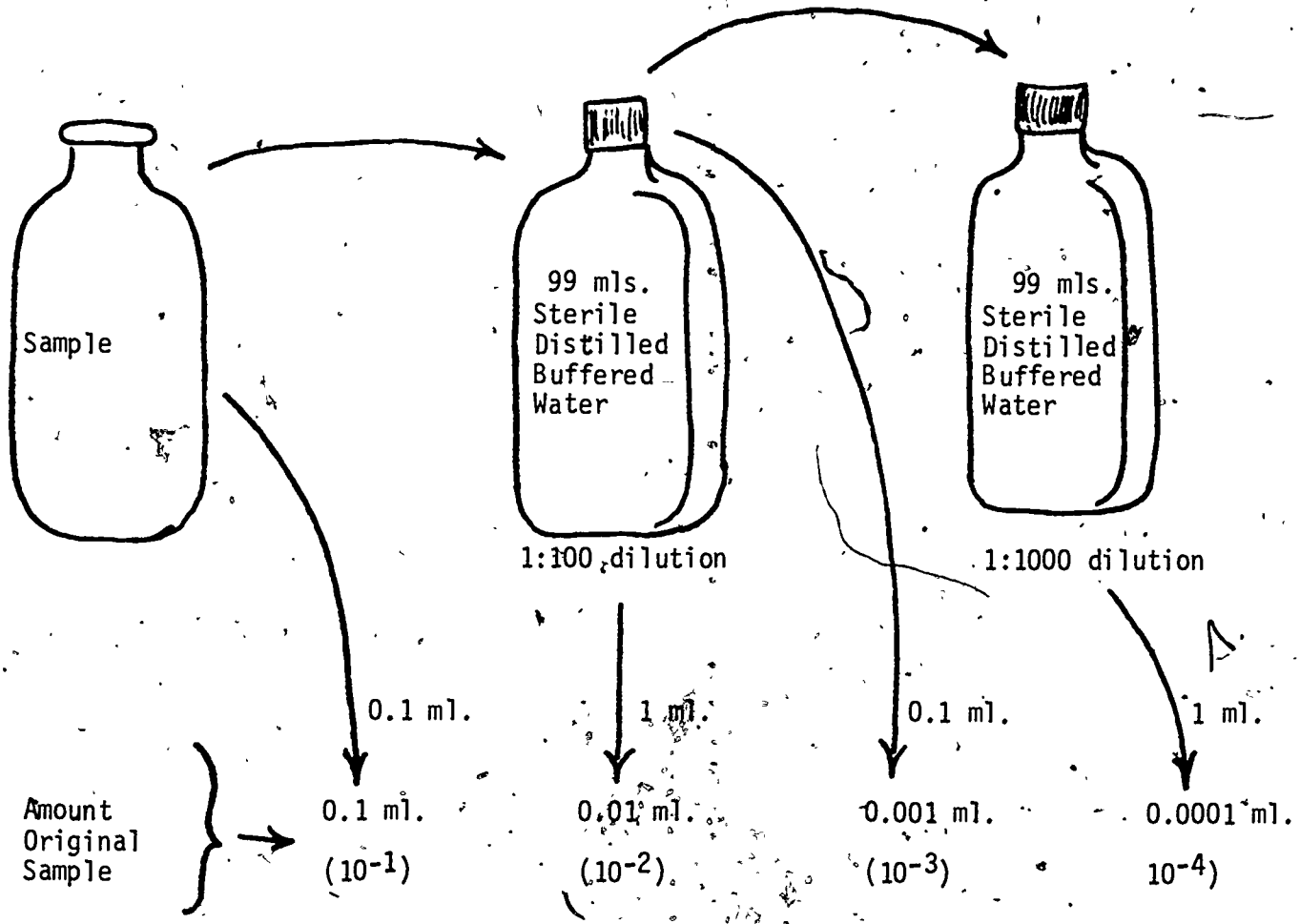
D. Sample not examined within 6 hrs. of collection. Bacteria will begin to die, so coliform determination will give counts which are lower than actual.

APPENDIX C - SAMPLE DILUTION

I. NECESSARY WHEN COUNT IS EXPECTED TO BE GREATER THAN 2,400 PER 100 ML

II. PROCEDURE

A.



B. Place 0.1 ml. sample into culture tube for 0.1 ml. dilution.

C. For 0.01 ml. sample volume

1. Place 1 ml. sample into a 99 ml. dilution blank.
2. Shake vigorously 25 times in an arc of 12"
3. 1 ml. of this 1:100 dilution represents 0.01 ml. of original sample.

D. For 0.001 ml. sample volume deliver 0.1 ml. from 1:100 dilution into the culture tube.

E. For 0.0001 ml. sample volume

1. Place 1 ml. of the 1:100 dilution into a fresh 99 ml. dilution blank.

2. Shake vigorously 25 times in an arc of 12"

3. 1 ml. of this 1:10,000 dilution represents 0.0001 ml. original sample volume.

F. For 0.00001 ml. sample volume deliver 0.1 ml. from the 1:10,000 dilution into the culture tube.

III. PRECAUTIONS

A. All volume measurement must be accurate

B. Any measurement error will be compounded in later steps

C. Transfer sample volumes aseptically because any contamination will be carried through entire process.

HANDOUT B

FECAL COLIFORM DETERMINATION IN WASTEWATER & WASTEWATER EFFLUENT

MEMBRANE FILTER TECHNIQUE

SECTION I: INTRODUCTION TO FECAL COLIFORM TESTING

I. FECAL COLIFORMS ARE A GROUP OF BACTERIA

A. Need oxygen to survive

B. Rod-shaped

C. Gram negative

D. Ferment the sugar lactose with gas formation within 24 hrs. at 44.5° C.

E. Colonies grow with a dark blue color on m-FC media within 24 hrs. at 44.5° C.

F. Found in fecal matter only.

G. See Figure #1 for size comparison

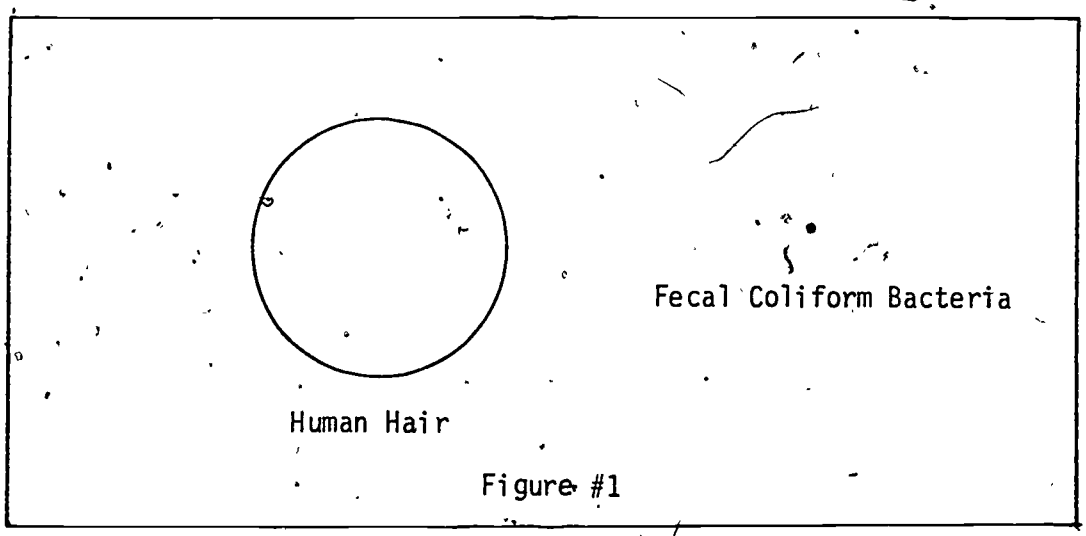


Figure #1

II. LARGE NUMBERS OF FECAL COLIFORMS IN WASTEWATER EFFLUENT MAY INDICATE

A. Untreated fecal matter present therefore disease causing organisms present.

B. Insufficient chlorination therefore disease causing organisms still alive also.

III. WATER QUALITY STANDARDS FOR WASTEWATER EFFLUENT & DISCHARGE WATERS

A. River or discharge water has maximum allowable fecal coliform level of 200/100 ml.

B. Sewage effluent has maximum allowable fecal coliform level of 400/100 ml.

SECTION 2: EQUIPMENT AND MEDIA PREPARATION

I. LIST OF BENCH EQUIPMENT, MEDIA, AND REAGENTS

A. Bench equipment

1. Hot plate
2. Balance with 0.5 gm. sensitivity
3. pH meter
4. Steriomicroscope (or other 10 x magnification device)
5. Round tipped forceps
6. Burner with open flame
7. Pipet soaking jar
8. Vacuum source

B. Glassware

1. 250 ml screw cap erlynmeyer flasks
2. Sample bottles
3. 100 ml. graduated cylinders
4. Filtering flasks
5. Membrane filter funnel
6. Reagent bottles
7. 4 oz. ointment jars
8. Re-pipettor with erlynmeyer flask
9. 100 ml. dilution bottles

C. Expendables

1. 10 ml. pipets
   a. Sterile, disposable cotton plugged, individually wrapped
   b. Or reusable with pipet can (to sterilize in)

2. 1 ml. pipets
   a. Sterile, disposable cotton plugged, individually wrapped
   b. Or reusable with pipet can (to sterilize in)
3. Membrane Filters
   a. 0.45 m pore rating
   b. 47 mm diameter
   c. White and gridded
   d. Sterile
4. Adsorbent pads
   a. High quality filter paper
   b. 48 mm in diameter
   c. Able to absorb 1.8 - 2.2 ml. of broth growth media
   d. Sterile
5. 50 x 12 mm sterile petri dishes with tight fitting covers
6. Non-adsorbant cotton
7. Cotton gauze
8. Brown Kraft wrapping paper
9. Aluminum foil
10. Rubber glvoes
11. Paper towels
12. Sponge

D. Safety Equipment
   1. Fire extinguisher
   2. Fire blanket
   3. First aid kit
   4. Emergency shower

E. Reagents and media

1. Rosalic Acid
2. m-FC broth or m-FC agar
3. Disinfectant
4. Peptone or $KH_2 PO_4$
5. 1 N NaOH

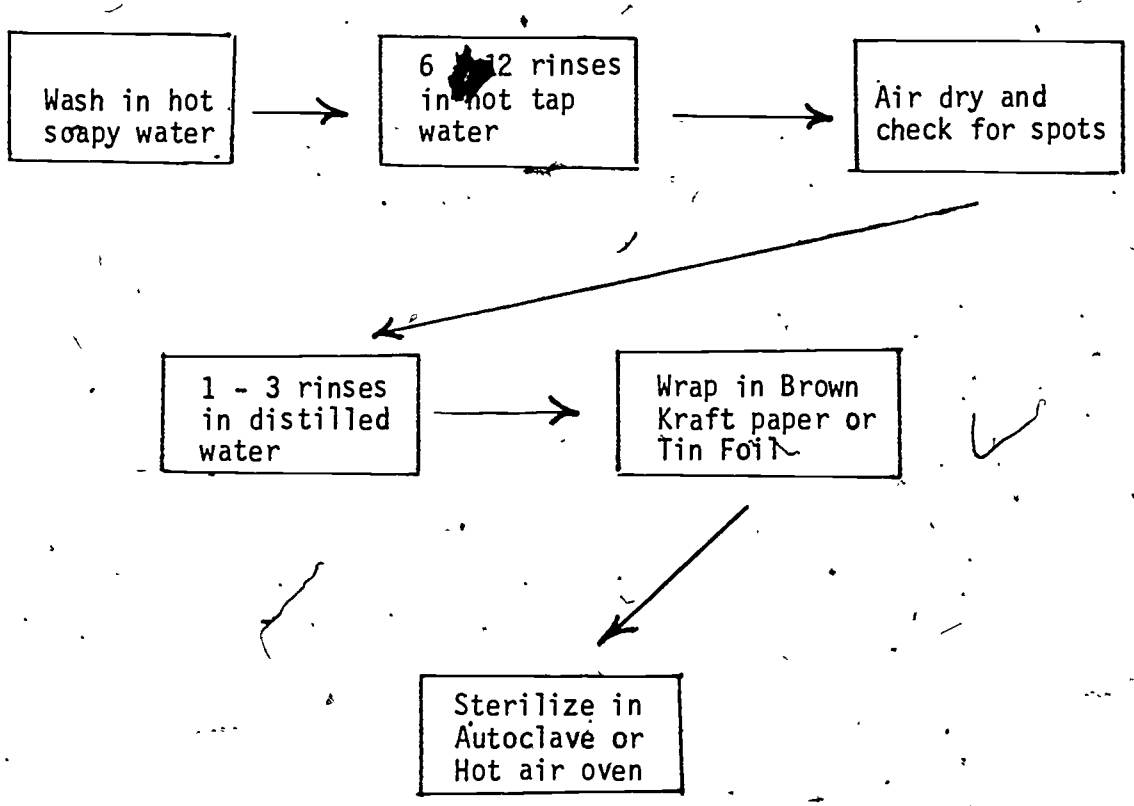
II. BENCH EQUIPMENT PREPARATION & FUNCTION

A. pH meter: Used to check pH of prepared media and reagents.

B. Stereomicroscope - 10 x 15 x: Used to count coliform colonies on membrane filters.

C. Balance with 0.5 gm sensitivity at 150 gms. to weigh media and reagents.

D. Filtration equipment & glassware preparation.



E. Function of Filtration Equipment & Glassware

1. Vacuum pump and tubing which must be able to pull 22" vacuum.

2. Trap flask which acts as safety trap to keep water out of pump.

3. Filtering flask.

   a. Traps water after it passes through filter.

   b. Must be sterilized as often as filtering funnel.

4. Filtering Funnel

   a. Seals membrane in place with no leaks.

   b. Available in stainless steel, corocillicate glass or autoclavable plastic.

   c. Need not be sterilized between consecutive filtrations.

   d. Must be sterilized if more than 1 hr. has elapsed since last sample filtration.

5. Round tipped forceps

   a. Use to handle membrane

   b. Must be free from rough or sharp edges

6. Sterile rinse bottle

   a. Filled with sterile distilled buffered rinse water

   b. Used to rinse inner surfaces of funnel between consecutive filtrations.

   c. i.e. erlynmeyer with repipettor

7. A 100 ml. graduated cylinder

   a. Used to measure water samples

   b. Must be sterile

   c. Must have 1 for each sample

8. Burner with open flame to ignite alcohol

   a. Bunsen burner

   b. Alcohol burner

F. Function of Expendable Equipment

1. Sterile 0.45 ms. membrane filters for water testing for trapping bacteria.

2. Sterile absorbant pads for holding media.

3. Sterile 1 ml. and 10 ml. pipets for measuring water samples.

III. REAGENT AND MEDIA PREPARATION

A. Use only distilled water

B. Sterile distilled buffered water - 2 types

1. Phosphate buffered water

a. Stock solution

1. Dissolve 34 gms. KH_2PO_4 in 500 mls. distilled water in volumetric flask.

2. Adjust pH to 7.2 with 1 N NaOH

3. Dilute 50 1 1. with distilled water

b. To make buffered water

1. Add 1.25 mls. stock to 1 1. distilled water

2. Mix, dispense and sterilize 20 min. at 121° C. (15 psi)

2. Peptone dillution water

a. Stock solution

1. Dissolve 10 gms. peptone in 100 mls. water

2. To store, sterilize 15 min. 121° C and store in refrigerator.

3. Discard if it becomes cloudy.

b. To make dilution water

1. Add 1 ml. stock solution per 100 mls. distilled water.

2. Mix, dispense, sterilize 20 min. at 121° C.(15 psi)

3. Sterilization and uses of distilled buffered water

a. Rinsing funnels between samples

1. Dispense and sterilize in autoclave 20 min. at 121° C. in cotton stoppered autoclavable rinse bottles.

2. Use slow exhaust.

3. Do not fill bottle over 3/4 full.

4. Sterilize delivery tube separately and aseptically assemble.

b. Dilution of samples

1. Dispense 99 mls. plus 4 mls. to allow for evaporation in 99 ml. dilution blanks.

2. Sterilize in autoclave 20 min. 15 121° C. (15 psi)

3. Use slow exhaust.

4. Sterilize with caps loose.

5. Tighten caps when removed from autoclave.

C. Sodium Thiosulfate Solution

1. Stock solution

a. Weigh 10 gms. of sodium thiosulfate

b. Dissolve in 50 - 60 mls. distilled water in a 100 ml. volumetric flask.

c. Add distilled water to bring to a final volume of 100 mls.

d. Transfer to stoppered, 100 ml. labeled bottle and store in refrigerator.

2. For use transfer 0.1 ml. stock solution (for each 4 oz. volume) to sample bottle before sterilization.

D. m-FC Preparation

1. Order in amounts to fit needs

a. Dehydrated broth media

1. 1 lb. bottle will make enough media for 4,000 filtrations.

2. ½ lb. bottle will make enough media for 1,000 filtrations.

b. Ampoules of prepared broth media

1. Can be ordered - 24 per package

2. Must be refrigerated and used within 1 year.

2. Prepare dehydrated m-FC media the day it is to be used.

3. Prepare dehydrated m-FC media according to manufacturers instruction.
   a. Do not overheat
   b. Do not sterilize
   c. Protect from light while cooling to room temperature
   d. Dispense when cool and use immediately.

SECTION 3: MEMBRANE FILTRATION PROCEDURE

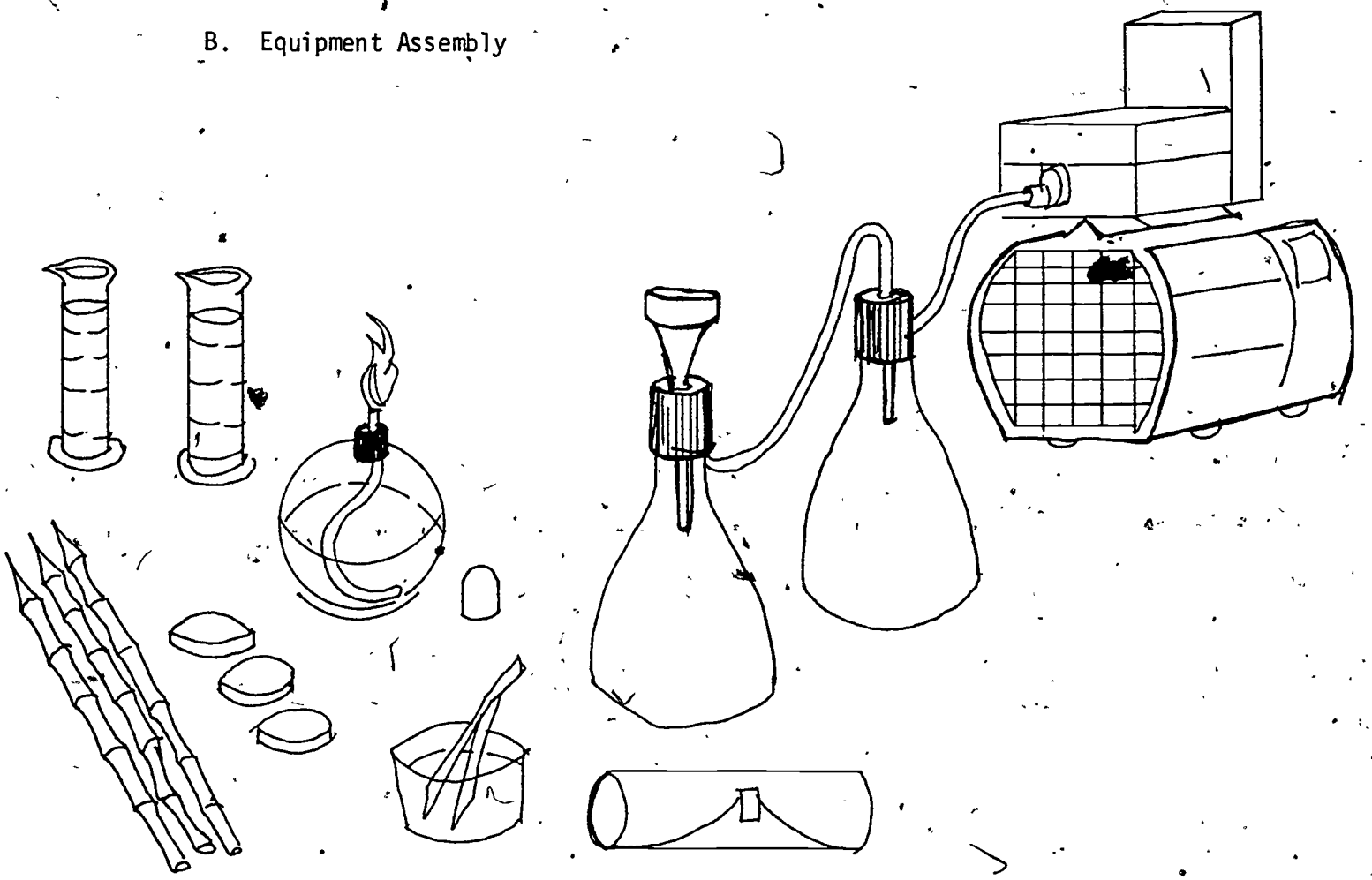
I. LABORATORY DATA SHEET PREPARATION

II. WORK AREA PREPARATION

A. Wash hands and disinfect work bench top

1. Lowers possibility of sample contamination leading to duplication of work.

B. Equipment Assembly



1. Connect vacuum tubing pump to trap flask.
2. Connect vacuum tubing trap flask to filtering flask.
3. Aseptically seal funnel base in vacuum flask.
4. Lay wrapped funnel in front.

5. Lay out burner forceps, alcohol jar, sterile M.F.'s sterile graduates, sterile pipets.

C. Dispense pads into 15 x 12 mm. petri dishes

1. From pack use flamed forceps.

2. From 100 pack use dispenser.

D. Dispense broth media

1. Use sterile 10 ml. pipet.

2. Dispense 1.8 to 2.2 ml. onto each pad.

3. Immediately before use decant excess media by gently tipping dish.

III. SAMPLE FILTRATION

A. Place membrane filter onto funnel base grid side up.

1. Membrane acts as trap for bacteria.

2. Membrane acts as support for colony growth.

B. Replace funnel top

C. Add sample and filter

1. If greater than 20 mls. just pour in.

2. If less than 20 mls. first pour in 20 mls. sterile distilled buffered water, then add sample volume to this.

3. Filter completely at 22" vacuum.

4. Rinse inner surfaces of funnel.

a. 3 separate rinses 20 mls. each.

b. Use sterile distilled buffered water.

c. Allow each rinse to filter completely before adding next.

d. This procedure rinses bacteria from inner surfaces of funnel.

1. Makes sterilization between consecutive samples unnecessary.

2. If more than 1 hr. elapses between samples re-sterilize unit.

D. Remove filter from unit

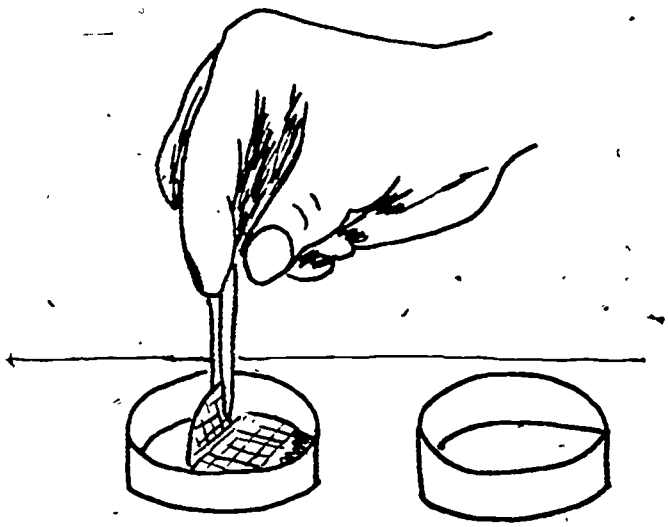
1. Carefully remove funnel top without disrupting membrane.

2. Dip forcep tips into alcohol and ignite to sterilize.

3. Pick up membrane with forceps touching only outer 1/8" inch of membrane.

IV. CULTURING MEMBRANE

A. Place membrane on saturated pad



1. Roll membrane to prevent air being trapped under membrane.

2. If air is trapped re-roll membrane.

3. Do not remove air by "smoothing with forceps".

4. Replace dish cover

B. Incubate cultured membrane

1. Invert dish

a. Membrane facing down

b. Keeps moisture in pad

c. Keeps moisture from dripping from lid onto membrane surface.

2. Incubate in a 44.5^{0} C ± 0.2^{0} C. incubator for 22 - 24 hours.

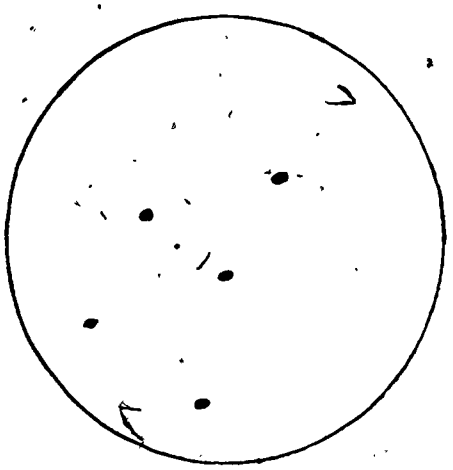
a. Use water bath or heat sink incubator with temperature control

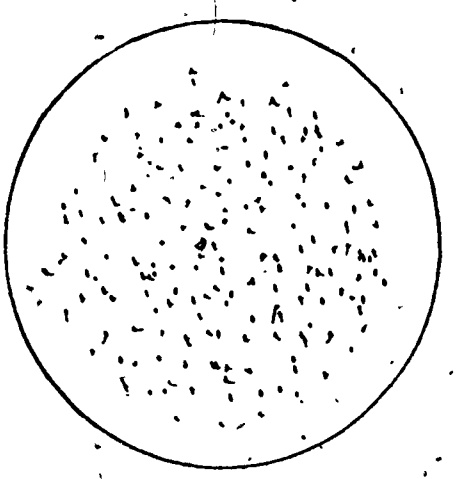
b. Allows fecal coliforms to multiply and form colonies.

c. Prevents growth of non-fecal coliform bacteria.

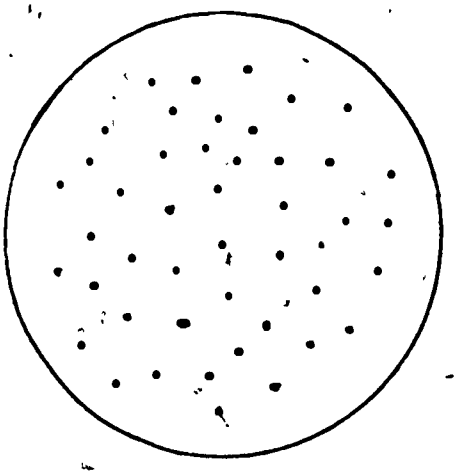
SECTION 4: MEMBRANE FILTER COUNTING PROCEDURE

I. COUNTING RANGE





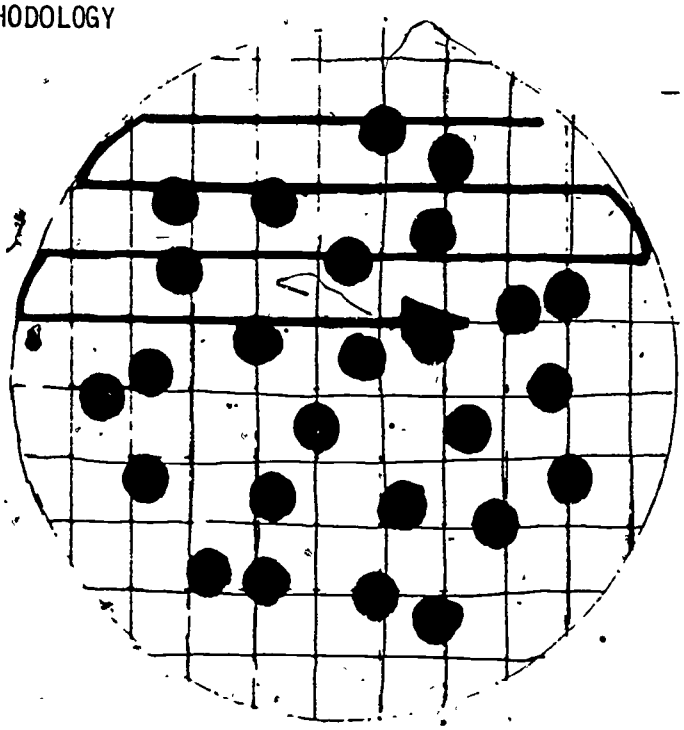
CHOOSE THE CORRECT MEMBRANE



A. 20 - 80 fecal coliform colonies

B. No more than 200 colonies total

II. COUNTING METHODOLOGY



A. Count colonies with the aid of the grid lines.

B. Count in a back and forth motion.

1. Count those colonies touching the top line.

2. Do not count those colonies touching the bottom line.

III. COLONY DIFFERENTIATION

A. Fecal coliform colonies are dark blue.

B. Non-coliform colonies are cream to gray in color.

SECTION 5: PROCESSING USED GLASSWARE

A. Contaminated but uncultured glassware

1. Sterilization unnecessary
2. Empty contents down drain
3. Wash, rinse, dry as previously described
4. Prepare for next testing series
   a. Prepare
   b. Wrap or package
   c. Sterilize

B. Glassware containing cultures

1. Sterilize in an autoclave
2. Empty contents down drain
3. Wash, rinse and dry as previously described
4. Prepare for next testing series
   a. Prepare
   b. Wrap, package, cap etc.
   c. Sterilize

C. Disposables

1. Discard in polypropylene bag
2. Sterilize in autoclave
3. Dispose of in garbage

SECTION 6: DATA INTERPRETATION AND EVALUATION

I. CALCULATION OF COUNT PER 100 ML.

Sample A	Sample B	Sample C
Amt. Filtered - Count	Amt. Filtered - Count	Amt. Filtered - Count
100 mls. 52	10 mls. TNTC	10 mls. 13
50 ml. 28	1 ml. 52	1 ml. 0
1 ml. 2	0.1 ml. 4	0.1 ml. 0
$\frac{\text{Count} + \text{Count}}{\text{Total Amt. filtered}} \times 100$	$\frac{\text{Count}}{\text{Amt. Filtered}} \times 100$	$\frac{\text{Count}}{\text{Amt. Filtered}} \times 100$
Example	Example	Example
$\frac{52 + 28}{100 + 50} \times 100 = 533$	$52/1 \times 100 = 5200$	$13/10 \times 100 = 130$
Report as: 530/100 mls.	Report as; 5200/100 mls.	Report as: 130/100 mls.

A. From Above Figure

1. Sample A - 2 counts within accepted range.
2. Sample B - 1 count within accepted range.
3. Sample C - Counts too low on all dilutions.

B. Counts too high on all dilution.

1. Report as TNTC (Too numerous to count)
2. Request new sample

C. Report all counts to 2 significant figures only.

1. i.e. report 392/100 ml. as 390/100 ml.

II. DATA EVALUATION

A. Sewage effluent

1. Maximum of 400 fecal coliforms per 100 mls.

B. Discharge water

1. Maximum of 200 fecal coliforms per 100 mls.

APPENDIX A - LABORATORY PREPARATION

I. SETTING LABORATORY RULES

A. Dress Code

1. Must wear lab coat or apron at all times.
2. Shoes must have full foot protection.
3. Long hair must be tied back.
4. Must wear protective clothing where applicable.
   a. Goggles or safety glasses
   b. Asbestos gloves

B. Safety Equipment

1. General Equipment
   a. Fire extinguisher
   b. Fire blanket
   c. First aid kit
   d. Emergency shower
   e. Emergency eye wash
2. Personal equipment for each employee
   a. Lab coat or apron
   b. Goggles
   c. Asbestos gloves
3. Safety rules
   a. Must be set and enforced by supervisor
   b. All accidents must be reported to supervisor

C. Record Keeping

1. Must be maintained at all times.
2. Should include all:

a. Purchase records

b. Equipment specifications, warranties, maintenance and instruction manuals.

c. Accident reports

d. Testing data

e. Pertinent communications

f. Employee records

II. LABORATORY CLEANLINESS

A. Types of disinfectants

1. 70% Ethanol

2. Phenols i.e. O-Syl

3. Quaterniary ammonium compounds

4. Halogen compounds

5. Activated sialdehyde i.e. cidex

B. Use of disinfectants

1. Weekly

a. Wipe down all shelves removing all glassware and books.

b. Wipe down all incubators, inside and outside.

c. Wipe out inside of autoclave.

2. Daily

a. Wipe down tops of all counters, large pieces of equipment

3. Immediately before testing disinfect work area.

4. Immediately disinfect spills.

C. Sources of Contamination

1. Dirt around lab.

2. Spilled samples or cultures.

3. Un-autoclaved bacterial garbage.

4. Chemical contamination from use of glassware for both Chemistry testing and Bacterial testing.

III. GLASSWARE WASHING

A. All glassware must be thoroughly washed in non-toxic detergent.

1. i.e. Alconox

2. Removes bacterial scum from glassware.

B. Rinse 6 - 12 times in hot tap water.

1. Removes detergent residue

2. Residue is harmful to bacteria

C. Final rinse 1 - 3 times in distilled water

1. Removes mineral residue from tap water.

2. Prevents water spotting.

D. Air Dry

1. Any spot indicates dirt

2. Rewash before using

IV. PACKAGING EQUIPMENT AND STERILIZATION

A. Reasons for packaging

1. Creates a bacteria barrier

2. Allows for storage of sterile equipment

B. Proper labeling

1. Define contents

2. Date to aid in equipment rotation

C. Sterilization of equipment - 2 Acceptable Methods

1. Autoclave

a. All rubber, metal and glassware and some plastics.

b. Normal cycle 15 min. 15 121^{0} C.

c. Exhaust rapidly

2. Hot air Sterilizing Oven

a. Dry glassware and metal objects only

b. Normal cycle 1 hr. at 170^{0} C.

c. Allow to cool before use

d. Package pipets in metal containers

e. Package other equipment with aluminum foil

V. MAJOR LABORATORY EQUIPMENT

A. Autoclave

1. Before using read and follow manufacturers installation use and maintenance instructions and safety precautions.

2. Normal sterilization = 15 psi yielding 121° C. for 15 min.

3. Use to sterilize liquids and non-heat sensitive equipment.

a. Most plastics are not autoclavable and sterilized by manufacturer.

b. Sterilized media and reagents must be removed from autoclave as soon as possible after autoclave is opened.

c. Glassware may be sterilized in autoclave but must be allowed to dry before removing from autoclave.

B. Hot Air Sterilizing Oven

1. Before using read and follow manufacturers installation, use, and maintenance instructions and safety precautions.

2. Normal Sterilization = 1 hour at 180° C.

3. Use to sterilize glass and metal only.

a. Rubber and plastics weill melt.

b. Liquids will evaporate and grow media components will be destroyed.

C. 44.5° C. Incubator

1. Before using, read and follow manufacturer's installation and maintenance instructions and safety precautions.

2. Design must be able to maintain a 44.5 ± 0.2° C. temperature tolerance.

a. Water bath type

b. Heat sink type

3. Place in permanent location

   a. Out of drafts and direct sunlight

   b. Convenient to laboratory bench and electrical outlet

4. Install thermometer

   a. NBS (National Bureau of Standards) certified thermometer

   b. Mercury bulb should be suspended in bottle filled with water

   c. Locate centrally in incubator

5. Adjust temperature to $44.5^{o} \pm 0.2^{o}$ C.

   a. Follow manufacturer's instructions

   b. Allow 1 hr. between temperature adjustments

   c. Record temperature of incubator daily

D. Water Distillation and Deionizing Unit

1. Before using, read and follow manufacturers installation, use and maintenance instructions and safety precautions.

2. Produces reagent grade water for use in making reagents and media and rinsing glassware.

3. May be used for preparation of water for both chemistry and bacteriology.

4. Store reserve distilled water in borosillicate glass or plastic carboys.

E. Refrigerator

1. Set to maintain a 4^{o} C. temperature.

2. Use to hold samples waiting to be tested and to store some prepared media and reagents.

F. Glassware washer

1. Before using, read and follow manufacturers installation, use and maintenance instructions and safety precautions.

2. Automatically washes and rinses glassware.

3. Do not use home dishwasher as it does not have proper plumbing.

APPENDIX B - COLLECTING SAMPLES FOR BACTERIOLOGICAL EXAMINATION

I. EQUIPMENT PREPARATION

A. Sample bottles must be:

1. At least 100 ml. capacity with a large neck opening.
2. Thoroughly cleaned with detergent, rinsed 6 times in hot tap water, rinsed finally in distilled-deionized water, then air dried.
3. Free from spots, scum, chips, cracks, excessive scratches and other damage on which bacteria may lodge.
4. Closed with preferably an all glass ground cap closure (but screw caps can be used providing liners are free from contamination and provide a non-leaking seal.
5. Sterilized in an autoclave at 121° C. for 15 min. with Kraft paper or tin foil hood covering caps and necks of bottles and slip of paper between bottleneck and glass stopper to prevent glass stopper from sticking.

B. Bottles intended for use in collection of chlorinated samples must have a 10% sodium thiosulfate solution added at the rate of 0.1 ml. for each 4 oz. bottle prior to sterilization and sterilized in bottle.

C. Labels must be:

1. Clean and unused.
2. Attached to bottle by a means not affected by water (i.e. string or wire.)

D. Label markers must be:

1. Permanent type not affected by water.
2. Able to mark on label.

E. Sampling devices must be in working condition and properly maintained.

F. Germicide must be available to clean up spills but must not come in contact with sample or any equipment touched by sample.

G. Rubber gloves must fit and not be punctured.

H. Ice chest for transporting sample must be:

1. Sufficient size to accommodate all samples.
2. Undamaged with tight cover so cold temperature can be maintained inside.

3. Filled with enough ice to quickly chill sample but little or no free water.

I. Refrigerator must be set at 2 - 10° C. and used if samples are not examined upon immediate return to lab.

II. SAMPLE COLLECTION

A. Minimum number of samples to be taken

1. Based on flow and industry on line.

B. To take sample from spigot or tap:

1. Find spigot with direct main connection

2. Put on rubber gloves.

3. Flush spigot at full flow for 2 - 3 min. to clear service line

4. If right handed, hold sample bottle near bottom with right hand and remove closure and paper hood with left hand (reverse if left handed). DO NOT LAY CLOSURE DOWN. Hold in such a way to protect closure and bottle from contamination.

5. Allow slip of paper between closure and bottle neck to fall to floor.

6. Thrust bottle into flowing water and allow bottle to fill about 3/4ths full. DO NOT RINSE, especially if bottle contains sodium thiosulfate to neutralize chlorine in sample.

7. Carefully replace closure and hood and secure.

8. Label bottle and place on ice in ice chest for transportation to laboratory.

C. To sample river, stream, lake, etc.

1. Put on rubber gloves.

2. If right handed, hold sample bottle near bottom with right hand and remove closure and paper hood with left hand (reverse if left handed). DO NOT LAY CLOSURE DOWN. Hold in such a way to protect closure and bottle from contamination.

3. Allow paper strip between and bottle to fall to ground.

4. To fill sample bottle

a. Turn bottle neck opening down and plunge below surface of water quickly to prevent dechlorinating agent from running out.

b. Turn upward to face bottle opening into current to avoid contamination of water flowing into bottle with samplers hand.

c. Allow to fill to about 3/4 full. DO NOT OVERFILL especially if bottle contains a dechlorinating agent.

d. Lift quickly out of water and replace closure and hood.

5. Label bottle and place on ice in ice chest for transportation to laboratory.

III. COMMON ERRORS AND AFFECT ON RESULTS

A. No dechlorinating agent in bottle. Chlorine activity continues until sample tested so bacteria continue to die and fecal coliform determination gives count which is lower than actual.

B. Sample not chilled when taken. Bacteria continue to multiply, so fecal coliform determination gives count which is higher than actual.

C. Bottle or closure contaminated. Extra bacteria introduced, so fecal coliform determination may give count which is higher than actual.

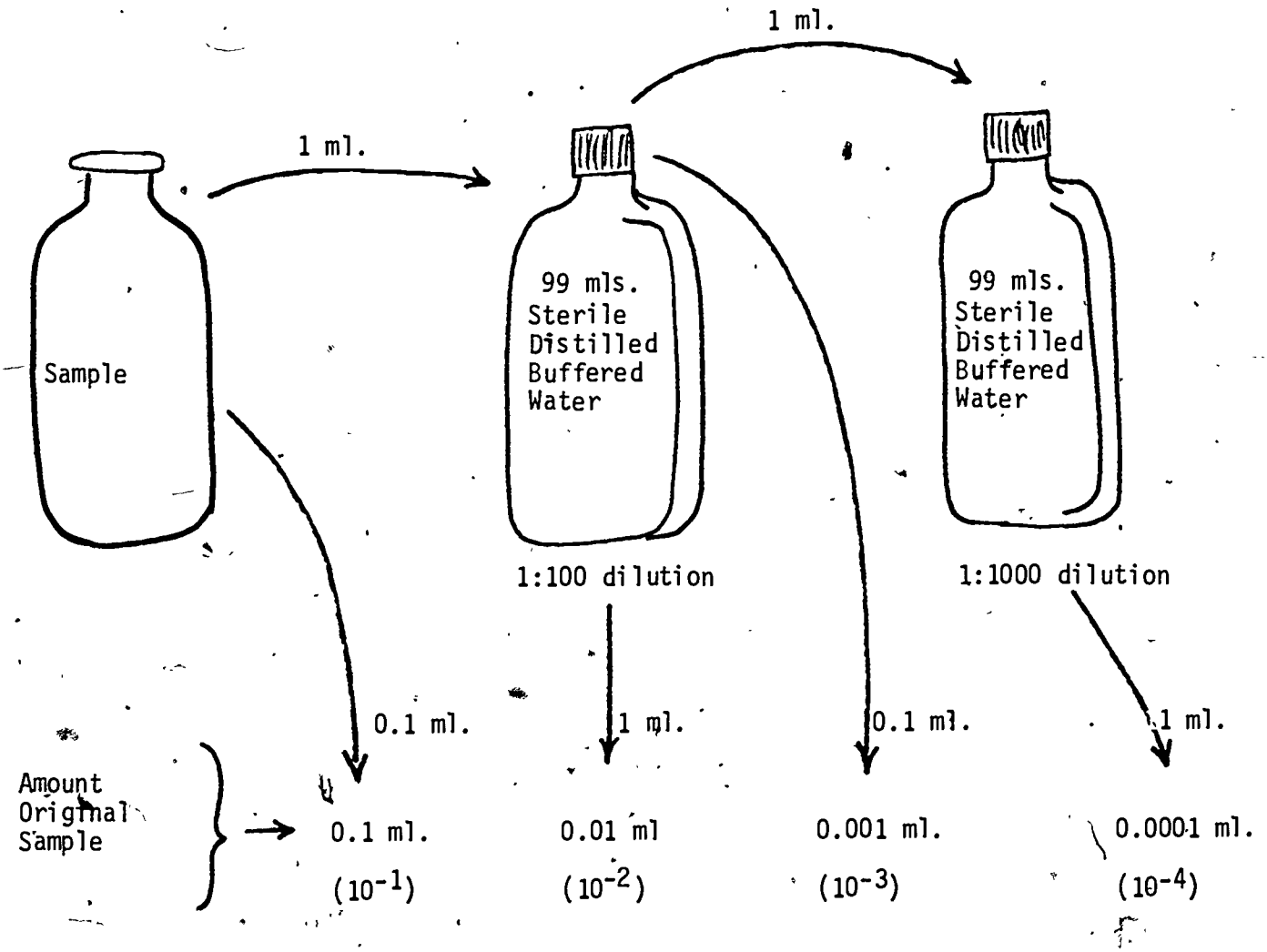
D. Sample not examined within 6 hrs. of collection. Bacteria will begin to die, so fecal coliform determination will give counts which are lower than actual.

APPENDIX C - SAMPLE DILUTION

I. NECESSARY WHEN COUNT IS EXPECTED TO BE GREATER THAN 8000 PER 100 ML

II. PROCEDURE

A.



B. Place 0.1 ml. sample into funnel for 0.1 ml. dilution.

C. For 0.01 ml. sample volume

1. Place 1 ml. sample into a 99 ml. dilution blank.
2. Shake vigorously 25 times in an arc of 12".
3. 1 ml. of this 1:100 dilution represents 0.01 ml. of original sample.

D. For 0.001 ml. sample volume deliver 0.1 ml. from 1:100 dilution into funnel.

E. For 0.0001 ml. sample volume

1. Place 1 ml. of the 1:100 dilution into a fresh 99 ml. dilution blank.
2. Shake vigorously 25 times in an arc of 12".
3. 1 ml. of this 1:10,000 dilution represents 0.0001 ml. original sample volume.

F. For 0.00001 ml. sample volume deliver 0.1 ml. from the 1:10,000 dilution into funnel.

III. PRECAUTIONS

A. All volume measurement must be accurate.

B. Any measurement error will be compounded in later steps.

C. Transfer sample volumes aseptically because any contamination will be carried through entire process.

FECAL COLIFORM DETERMINATION IN WASTEWATER & WASTEWATER EFFLUENT

Transparancy List

Transparancy #1: Sample dilution

Transparancy #2: MPN equipment

Transparancy #3: Pipet and loop

Transparancy #4: Positive test

Transparancy #5: Recording MPN test data

Transparancy #6: MPN chart

Transparancy #7: MF equipment

Transparancy #8: MF equipment-set up

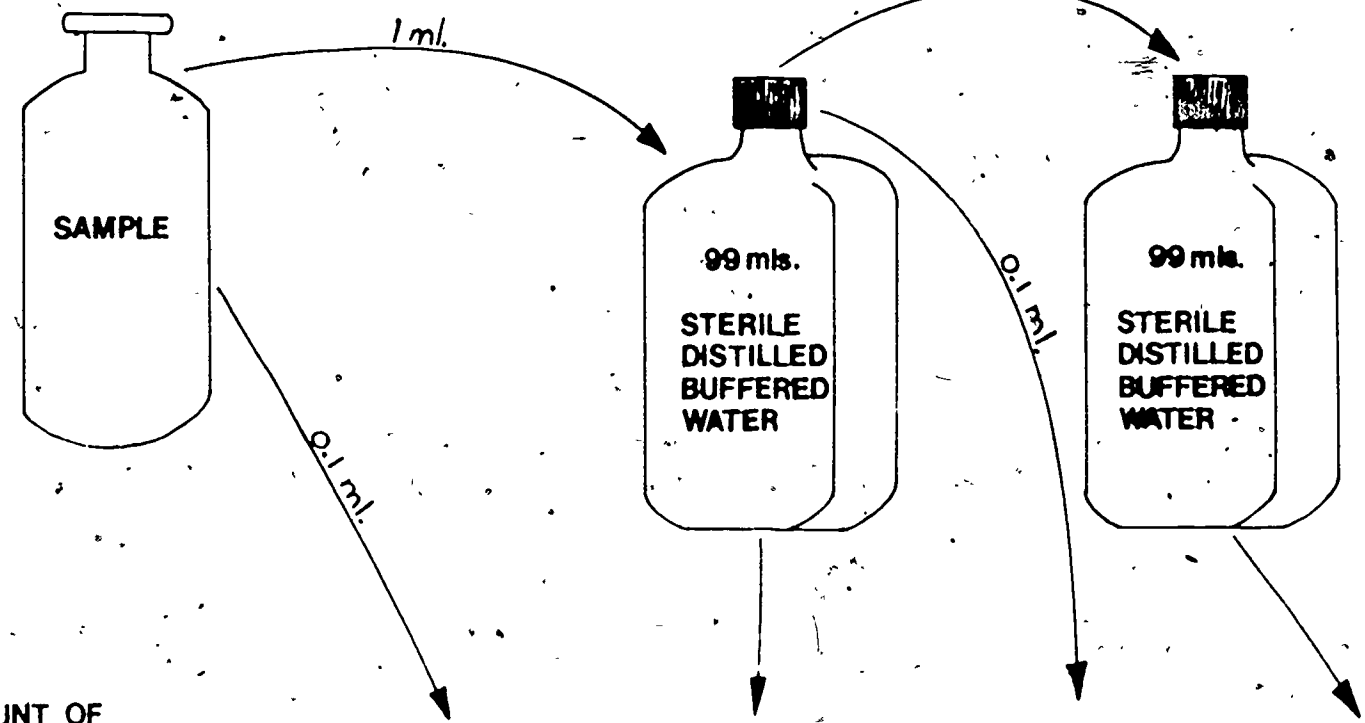
Transparancy #9: Plating method

Transparancy #10: - Choose correct MF to count

Transparancy #11: Counting methodology

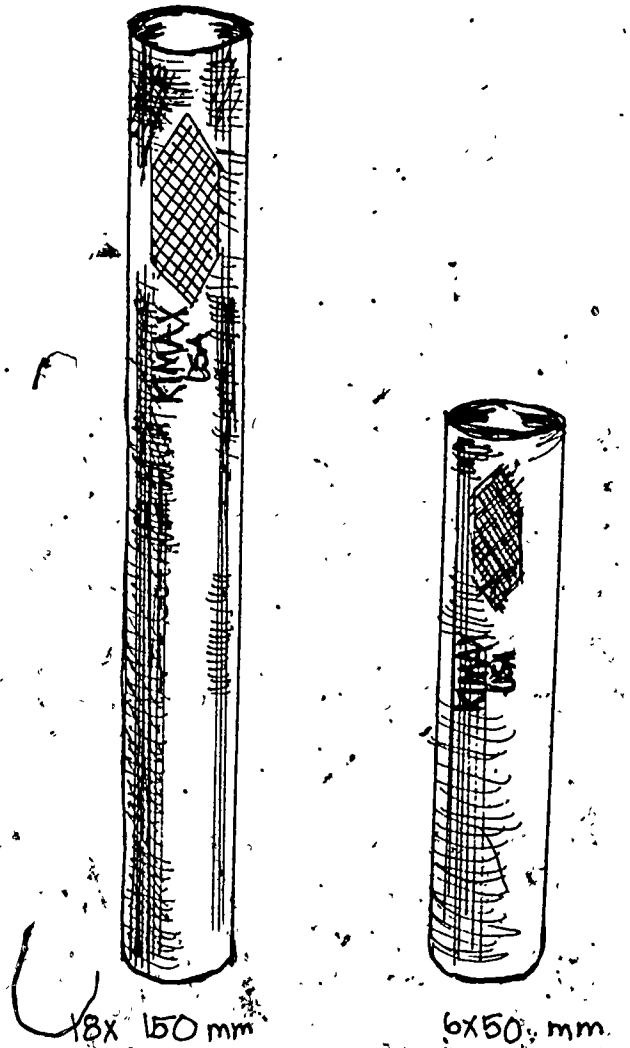
Transparancy #12: Calculating count per 100 mls.

SAMPLE DILUTION

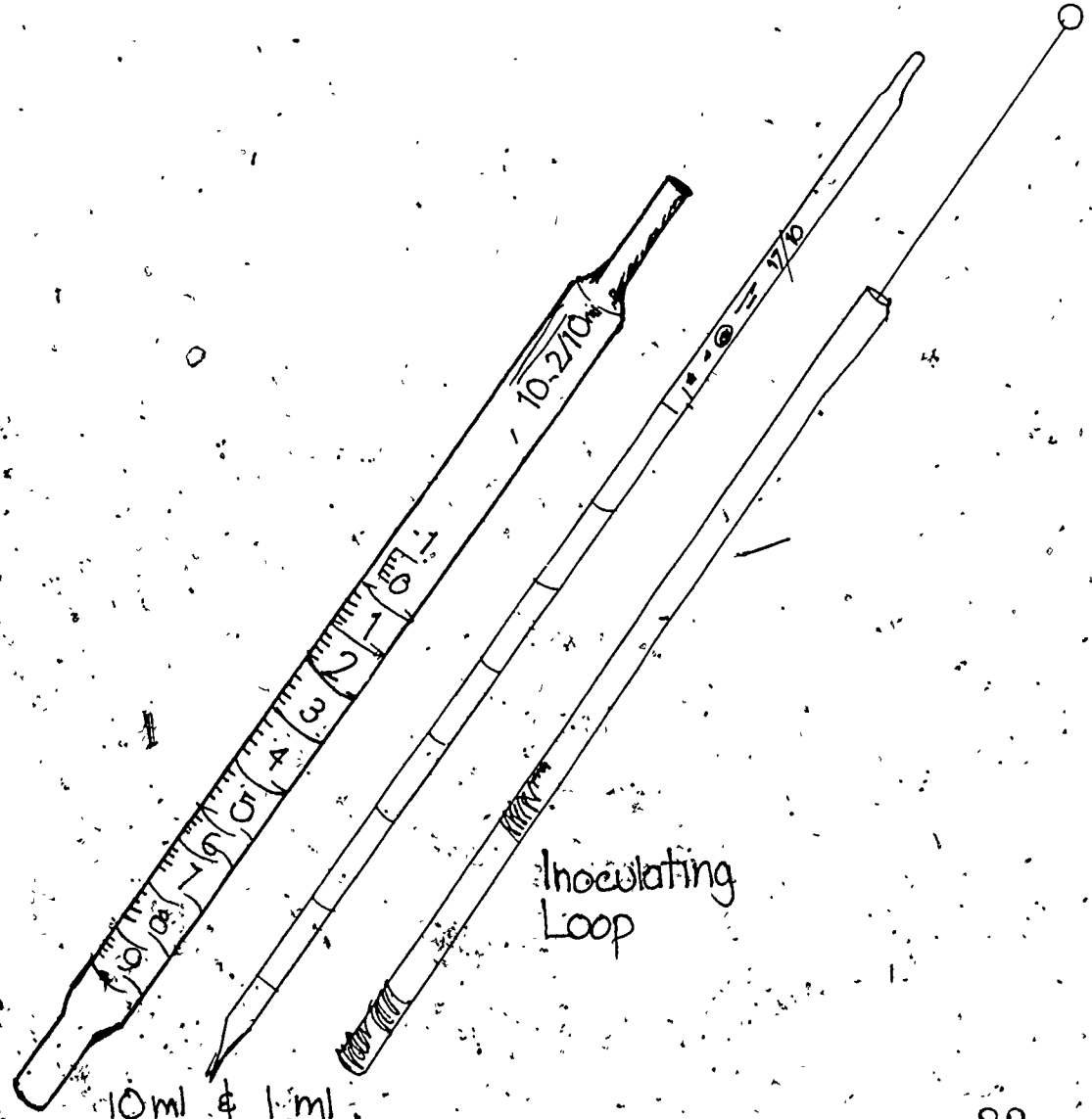


AMOUNT OF ORIGINAL SAMPLE :	1/10 ml. 0.1 ml.	1/100 ml. per ml. 0.01 ml. per ml.	1/1000 ml. 0.001 ml.	1/1000 ml. per ml. 0.0001 ml. per ml.
	10^{-1}	10^{-2}	10^{-3}	10^{-4}

Multiple Tube Technique Equipment

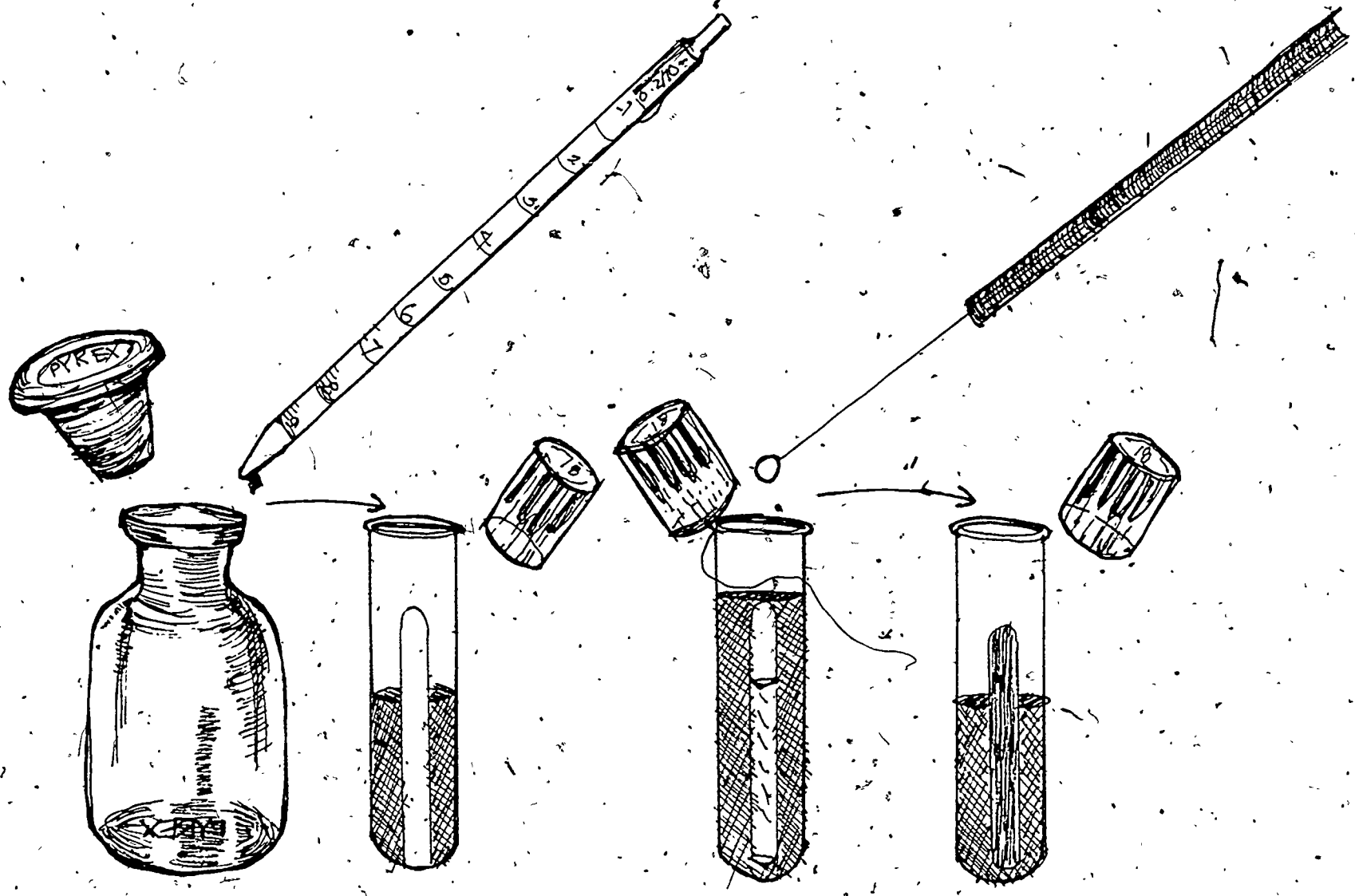


Test Tubes



10ml & 1ml Sterile Pipets

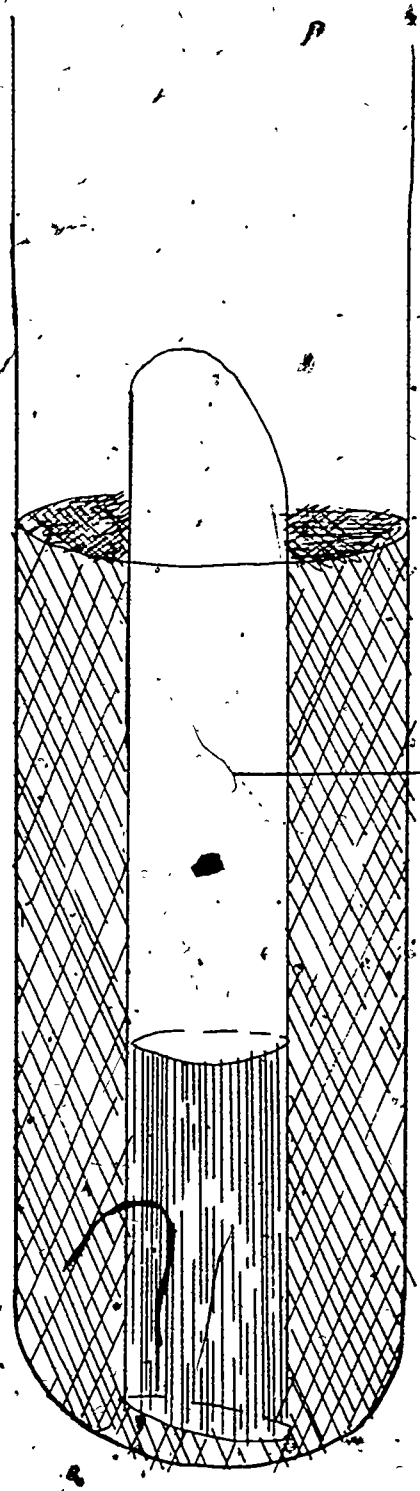
Use of PIPET and LOOP



Use Pipet to inoculate presumptive media with sample.

Use loop to transfer growth from positive presumptive media to confirmed media.

Positive Test



Trapped Gas Produced by growing Coliforms

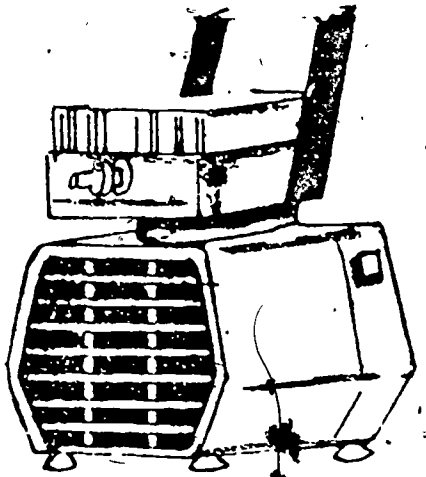
MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS WHEN VARIOUS NUMBERS OF RUBES ARE USED PER DILUTION (10 ML, 1.0 ML, 0.1 ML)

Combination of Positives	Tubes Per Dilution: 3 — MPN Index /100 ml	3 — 95% Confidence Limits: Lower	3 — 95% Confidence Limits: Upper	5 — MPN Index /100 ml	5 — 95% Confidence Limits: Lower	5 — 95% Confidence Limits: Upper
0-0-0	3			2		
0-0-1	3	0.5	9	2	0.5	7
0-1-0	3	0.5	13	2	0.5	7
0-2-0				4	0.5	11
1-0-0	4	0.5	20	2	0.5	7
1-0-1	7	1	21	4	0.5	11
1-1-0	7	1	23	4	0.5	11
1-1-1	11	3	36	6	0.5	15
1-2-0	11	3	36	6	0.5	15
2-0-0	9	1	36	5	0.5	13
2-0-1	14	3	37	7	1	17
2-1-0	15	3	44	7	1	17
2-1-1	20	7	89	9	2	21
2-2-0	21	4	47	9	2	21
2-2-1	28	10	150	12	3	28
2-3-0						
3-0-0	23	4	120	8	1	19
3-0-1	39	7	130	11	2	25
3-0-2	64	15	380			
3-1-0	43	7	210	11	2	25
3-1-1	75	14	230	14	4	34
3-1-2	120	30	380			
3-2-0	93	15	380	14	4	34
3-2-1	150	30	440	17	5	46
3-2-2	210	35	470			
3-3-0	240	36	1,300			
3-3-1	460	71	2,400			
3-3-2	1,100	150	4,800			
3-3-3	2,400					
4-0-0				13	3	31
4-0-1				17	5	46
4-1-0				17	5	46
4-1-1				21	7	63
4-1-2				26	9	78
4-2-0				22	7	67
4-2-1				26	9	78
4-3-0				27	9	80
4-3-1				33	11	93
4-4-0				34	12	93
5-0-0				23	7	70
5-0-1				31	11	89
5-0-2				43	15	110
5-1-0				33	11	93
5-1-1				46	16	120
5-1-2				63	21	150
5-2-0				49	17	130
5-2-1				70	23	170
5-2-2				94	28	220
5-3-0				79	25	190
5-3-1				110	31	250
5-3-2				140	37	340
5-3-3				180	44	500
5-4-0				130	35	300
5-4-1				170	43	490
5-4-2				220	57	700
5-4-3				280	90	850
5-4-4				350	120	1,000
5-5-0				240	68	750
5-5-1				350	120	1,000
5-5-2				540	180	1,400
5-5-3				920	300	3,200
5-5-4				1,600	640	5,800
5-5-5				2,400		

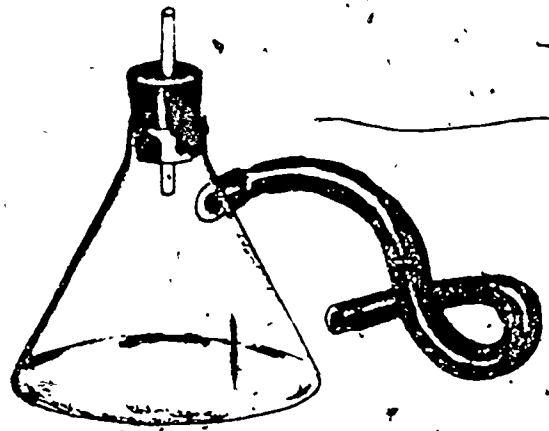
RECORDING MPN DATA

SAMPLE NUMBER	TUBE NUMBER	VOLUME INOCULATED	DATE INOCULATED	PRESUMPTIVE RESULTS		CONFIRMATORY RESULTS 48 HR.	TECHNICIAN'S INITIALS
				24 HR.	48 HR.		

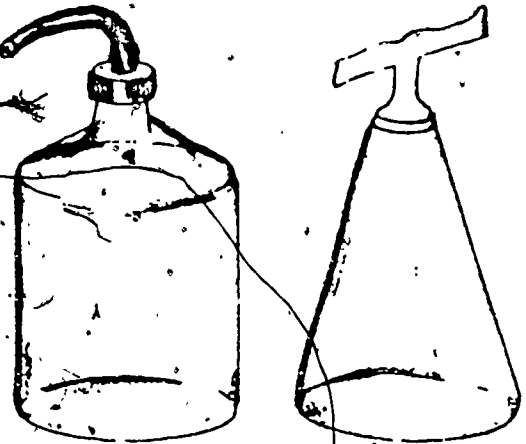
Membrane Filtration Equipment



Vacuum pump



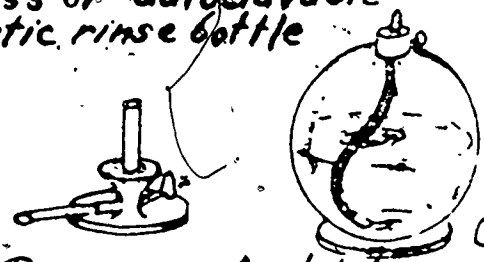
Trap flask
Filtering flask
Vacuum tubing



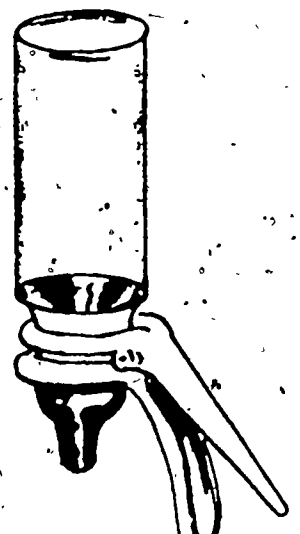
Glass or autoclavable plastic rinse bottle

Forceps

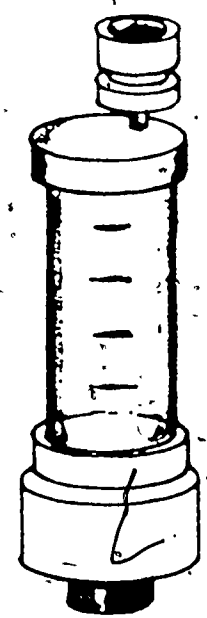
100 ml graduated cylinder

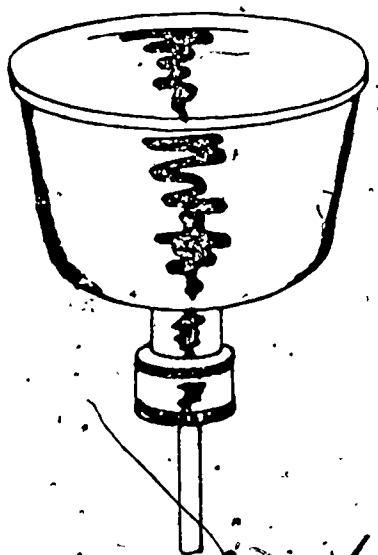


Bunsen or Alcohol burner

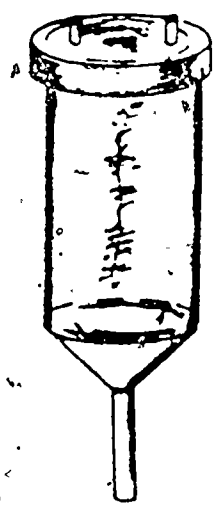


Filtering funnel. Borosilicate glass Rubber collar or clamp coupling



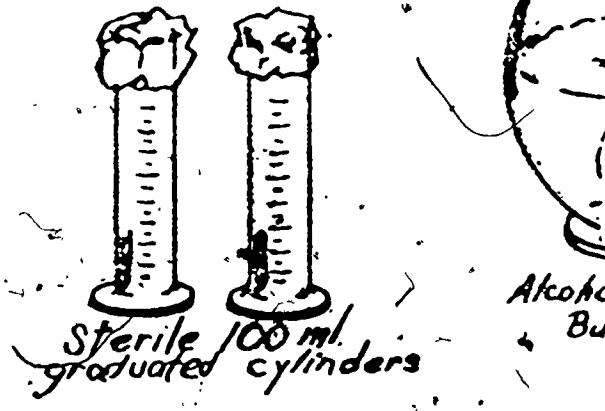


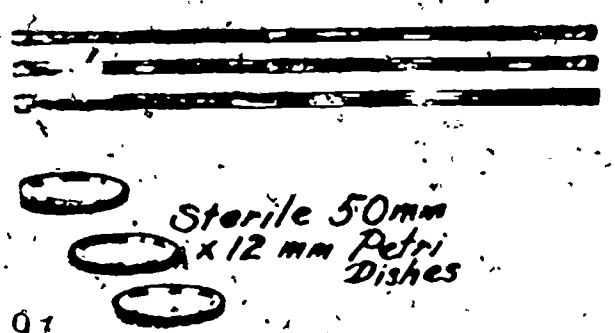
Filtering funnel Stainless steel twist coupling

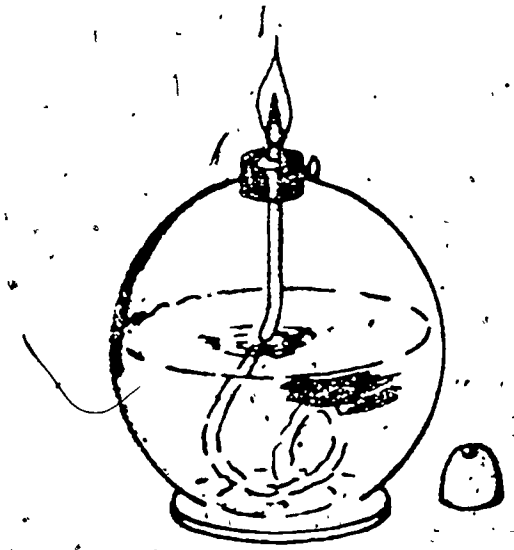


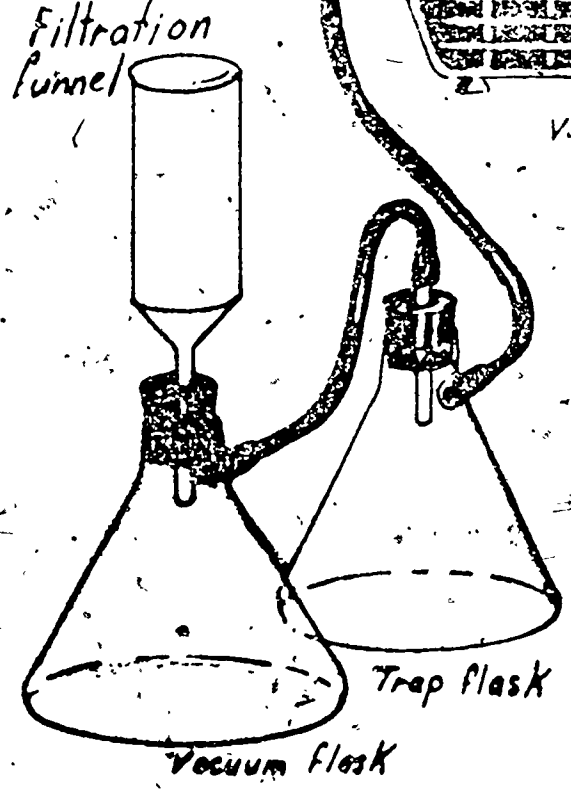
Filtering funnel Polysulfone plastic magnetic coupling

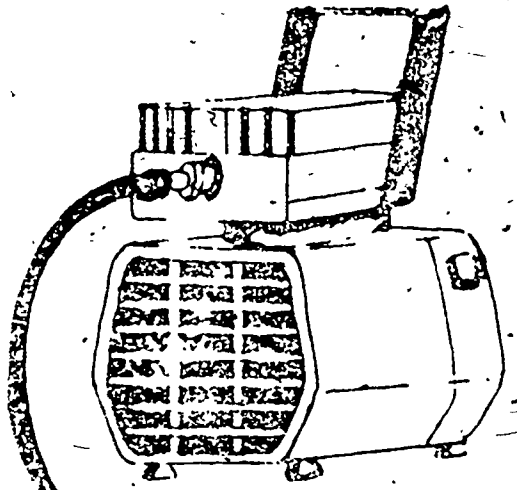
Membrane Filtration Equipment Assembly

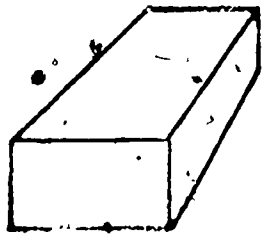


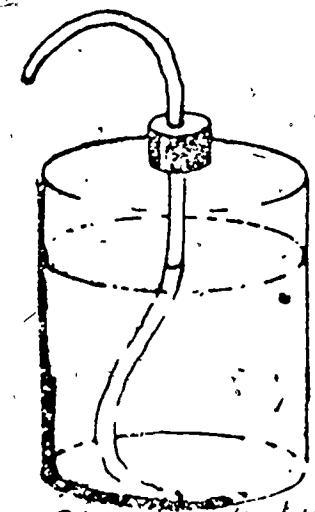




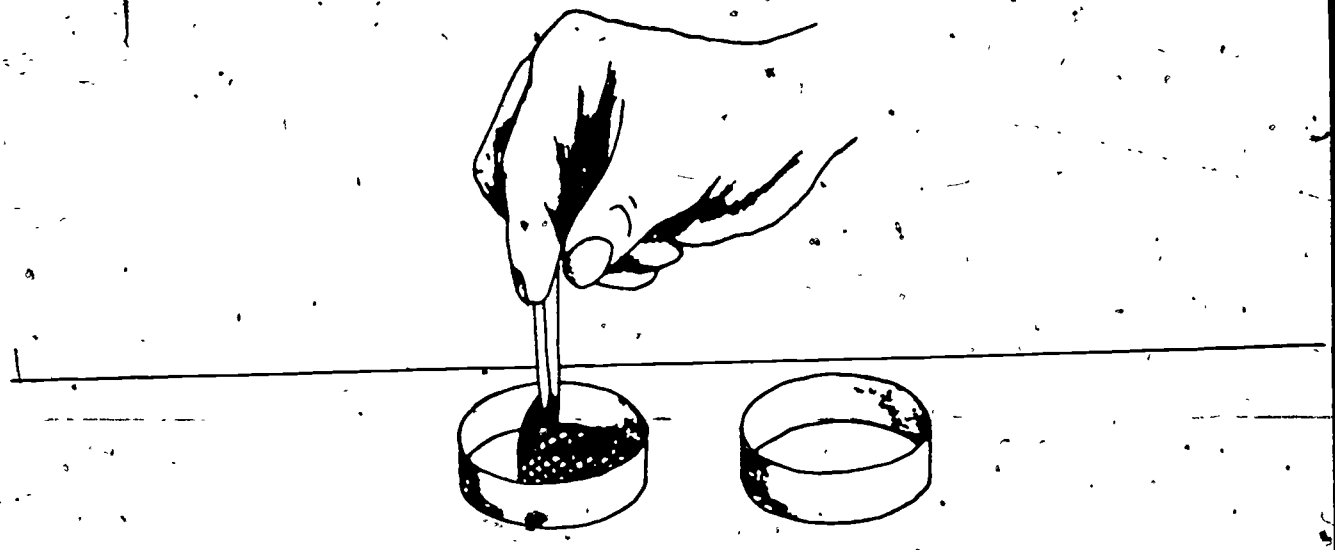






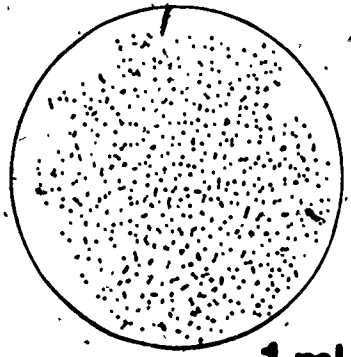


PLACEMENT OF MEMBRANE ONTO PLATE OF MEDIA

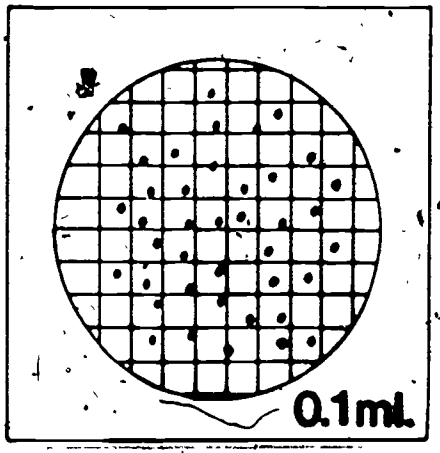


FROM: SIMPLIFIED PROCEDURES FOR WATER EXAMINATION - LAB. MANUAL AWWA

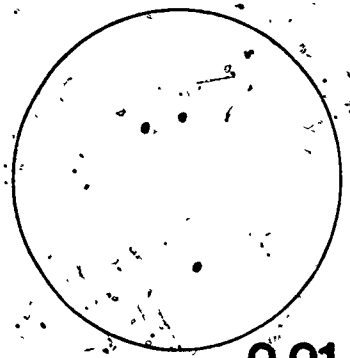
CHOOSE THE CORRECT MEMBRANE



1 ml.



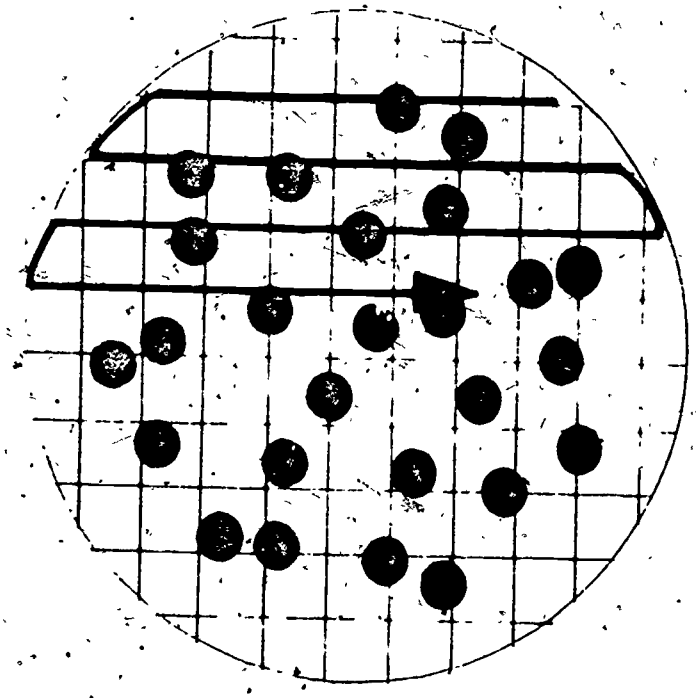
0.1 ml.



0.01 ml.

CONTAINS 20 - 80 COLIFORM COLONIES, BUT FEWER THAN 200 COLONIES TOTAL.

Membrane Filter Counting Procedure



CALCULATIONS

Sample A

Amt. Filtered	Count
100 mls.	52
50 ml.	28
1 ml.	2

$$\frac{\text{Count} + \text{Count}}{\text{Total Amt. filtered}} \times 100$$

Example

$$\frac{52 + 28}{100 + 50} \times 100 = 533$$

Report as: 530/100 mls.

Sample B

Amt. Filtered	Count
10 mls.	TNTC
1 ml	52
0.1 ml.	4

$$\frac{\text{Count}}{\text{Amt. Filtered}} \times 100$$

Example

52/1 x 100 = 5200

Report as: 5200/100 mls.

Sample C

Amt. Filtered	Count
10 mls.	13
1 ml.	0
0.1 ml.	0

$$\frac{\text{Count}}{\text{Amt. filtered}} \times 100$$

Example

13/10 x 100 = 130

Report as: 130/100 mls.

Module No:	Module Title: Fecal Coliform Determination in Wastewater & Wastewater Effluent
Approx. Time:	Submodule Title: Multiple Tube Technique
	EVALUATION - Part A

Objectives:

Upon completion of this module, the participant should be able to demonstrate the ability to perform a total coliform determination by the multiple tube technique and/or accurately answer 80% of the evaluation questions over the procedure

EXAM QUESTIONS

Topic: Introduction

1. What does the presence of excessive numbers of fecal coliforms in wastewater effluent indicate with respect to chlorination?
2. If large numbers of fecal coliform bacteria are present in wastewater effluent what other type of organisms of concern may also be present?
3. Describe the fecal coliform group with respect to the following characteristics:
   a. _____ shaped
   b. gram _____
   c. Produce _____ when ferments lactose.

Topic: Laboratory Equipment

1. What would an autoclave be used for?
2. Why use an incubator for growing bacteria?
3. State 2 reasons for using only a cotton plugged, sterile pipet when pipeting by mouth in a microbiology lab.
4. Why is it important to properly rinse glassware after washing?
5. What device is used to test the pH of growth media?
6. What device is used to transfer the bacteria from the positive presumptive test into the confirming media?

Topic: Laboratory and Media Preparation

1. State the 2 things a disinfectant is used for.
2. Why is the sample bottle wrapped before being sterilized?
3. State 2 ways to obtain distilled water.
4. What are the 2 chemicals that can be used to buffer sterile dilution water?
5. Is the growth media for this procedure sterilized in a hot air oven or atoclave?
6. What would an autoclave cycle of 15 min. at 121° C (15 psi) with a rapid exhaust and a 10 min. allowance for drying be used for?
7. Where is sterile distilled buffered water stored.
8. Why must paper wrapped equipment remain dry after sterilization?
9. List the steps in proper glassware washing.
10. Is tap water of sufficient quality to use for growth media preparation?

Topic: Sampling

1. What chemical is used to dechlorinate a sample?
2. Why is a sampling tap flamed with a propane torch?
3. How long may a sample be held before it is tested for fecal coliform bacteria?
4. What happens to the bacterial population if the sample is not kept chilled?

Topic: Sample Dilution

1. Should sample dilution ever be necessary when sampling wastewater effluent?
2. Diagram how to get a 1:10000 dilution.

Topic: Multiple Tube Test Procedure and Data Interpretation

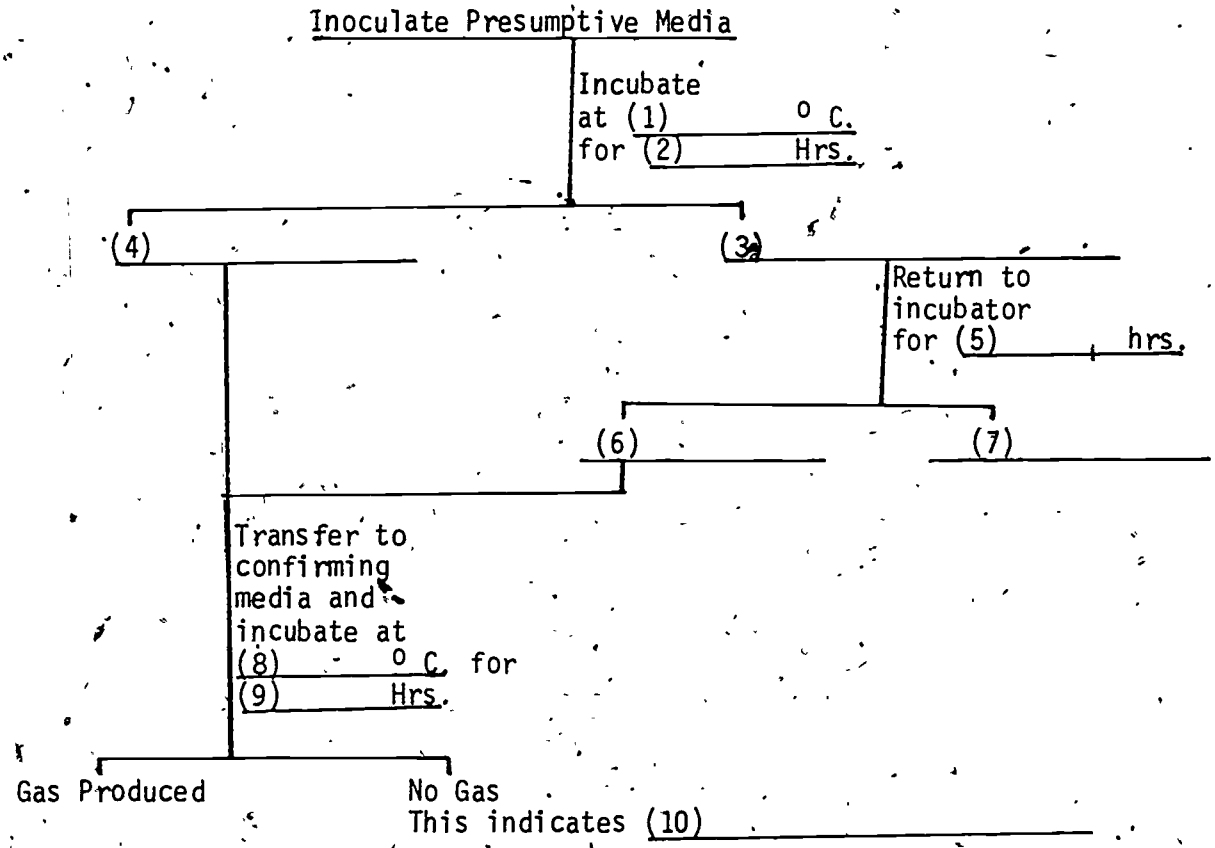
1. Why is the work area disinfected immediately before testing begins?
2. Which broth is inoculated directly with the water sample Lauryl Tryptose Sulfate or E. C. broth?

3. At what temperature are the inoculated tubes incubated for the confirming test?

4. Is it necessary to use aseptic technique in planting the sample in the presumptive growth media transferring growth to the confirming media?

5. How are the old cultures processed before test tubes are washed?

6. Fill in the ten blanks with the correct information.



7. What is the fecal coliform MPN Index per 100 ml for the following test data?

Sample No.	Tube No.	Volume Inoculated	Date Inoculated	Presumptive Results 24 Hr.	48 Hr.	Confirmed Results	Tech. Initials
1039	1A	0.1 ml	3/10	-	+	+	
1039	1B	0.1 ml	3/10	-	+	+	
1039	1C	0.1 ml	3/10	-	-		
1039	2A	1 ml	3/10	+		+	
1039	2B	1 ml	3/10	-	+	-	
1039	2C	1 ml	3/10	-	-		
1039	3A	10 ml	3/10	+		+	
1039	3B	10 ml	3/10	-	+	+	
1039	3C	10 ml	3/10	+		+	

Combination of Positives 3 Tubes Per Dilution	MPN Index /100 ml	95% Confidence Limits Lower	Upper
0-0-0	3		
0-0-1	3	0.5	9
0-1-0	3	0.5	13
0-2-0			
1-0-0	4	0.5	20
1-0-1	7	1	21
1-1-0	7	1	23
1-1-1	11	3	36
1-2-0	11	3	36
2-0-0	9	1	36
2-0-1	14	3	37
2-1-0	15	3	44
2-1-1	20	7	89
2-2-0	21	4	47
2-2-1	28	10	150
2-3-0			
3-0-0	23	4	120
3-0-1	39	7	130
3-0-2	64	15	380
3-1-0	43	7	210
3-1-1	75	14	230
3-1-2	120	30	380
3-2-0	93	15	380
3-2-1	150	30	440
3-2-2	210	35	470
3-3-0	240	36	1,300
3-3-1	460	71	2,400
3-3-2	1,100	150	4,800
3-3-3	2,400		

Module No:	EVALUATION - PART A.

Instructor Notes:	Instructor Outline:
Answers Topic: Introduction 1. Chlorination has been insufficient. 2. Disease causing micro-organisms. 3. a. Rod b. Negative c. Gas Topic: Laboratory Equipment 1. Sterilizing heat stable equipment and liquids. or Processing old cultures before disposal. 2. It provides a controlled environment for the bacteria to grow in. 3. To protect the sample from contamination. To protect the lab technician from contamination. 4. Rinsing removes detergent residue which can inhibit bacterial growth. 5. a pH meter 6. a 3 mm. inoculating loop	Give the participants a sample to analyze by the multiple tube method and/or the total coliform - multiple tube evaluation questions to answer.

Module No:	EVALUATION - PART A.

Instructor Notes:	Instructor Outline:

Topic: Laboratory & Media Preparation

1. General Laboratory Cleanup
   Cleaning up spilled bacterial cultures.

2. It allows the sample bottle to be stored without becoming contaminated.

3. Purchase a distillation unit and make it or purchase the distilled water from a reliable source.

4. Peptone
   $KH_2 PO_4$ (Potassium dihydrogen phosphate)

5. Autoclave

6. Sterilizing dry goods (i.e. glassware)

7. In the refrigerator

8. Bacteria is able to move through wet paper to contaminate the contents but not through dry paper.

9. 1. Wash in hot soapy water.
   2. Rinse in hot tap water 6 - 12 times
   3. Rinse 1 - 3 times in distilled water
   4. Air dry
   5. If spots appear when dry, rewash.

10. No

Topic: Sampling

1. Sodium thiosulfate

2. To incinerate the bacteria on it.

Module No:	EVALUATION - PART A

Instructor Notes:	Instructor Outline:

3. 6 hours

4. It will change first with growth followed by rapid die off.

Topic: Sample Dilution

1. No

2. Sample 1 ml → 99 ml dilution blank
   ↓ 1 ml
   99 ml dilution blank ←
   This is the 1:10,000 dilution

Topic: Multiple Tube Test Procedure & Data Interpretation

1. Disinfection removes most dust and bacteria from the work area and this lowers the risk of contamination.

2. Lauryl Tryptose Sulfate Broth

3. 44.5 ± 0.2° C

4. Yes

5. They are sterilized in an autoclave.

6. (1) 35 ± 0.5° C
   (2) 24 - 48 hrs.
   (3) No gas
   (4) Gas produced
   (5) 24 hrs.
   (6) Gas produced
   (7) No gas
   (8) 44.5 to 2° C
   (9) 24 Hrs.
   (10) No fecal coliforms present

7. 120 fecal coliforms/100 mls.

Module No:	Module Title:
	Fecal Coliform Determination of Water and Wastewater Effluent
	Submodule Title:
Approx. Time:	M. F. Technique
	EVALUATION - PART B

Objectives:

Upon completion of this module, the participant should be able to demonstrate the ability to perform a fecal coliform determination by the membrane filter technique and/or accurately answer 80% of the evaluation questions over the procedure.

EXAM QUESTIONS

Topic: Introduction

1. What does the presence of large numbers of fecal coliforms in the final chlorinated effluent?

2. Why is chlorination not required during the winter months for some waste treatment plants?

3. Describe the fecal coliform group with respect to the following characteristics:

   a. Shape
   b. Gram _____
   c. Found in _____ _____ only differentiated from other coloform bacteria.
   d. By their ability to grow at _____ ^{o} C.
   e. Colonies grow with a _____ color on MFC media

4. Using the membrane filter technique the fecal coliform monthly average must not exceed _____ per _____ mls. in order for the effluent to meet current standards.

Topic: Laboratory Equipment

1. What would an autoclave be used for?

2. Is a 44.5^{o} C. ± 0.2^{o} C. incubator usually a circulated water bath incubator or a circulated warm air incubator?

3. List the 5 pieces of equipment in use when filtering a sample.

4. State 2 reasons for using only a cotton plugged, sterile pipet when pipetting by mouth in a microbiology lab.

5. Why is it important to properly rinse glassware after washing?

6. Why must bacterial cultures be sterilized before being disposed of?

Topic: Laboratory Preparation

1. State the 2 things a disinfectant is used for.
2. Why is equipment packaged or wrapped before being sterilized?
3. State 2 ways to obtain distilled water.
4. What are the 2 chemicals that can be used to buffer sterile dilution water?
5. Is m-FC growth media sterilized and why?
6. Where is the 1% Rosalic Acid solution stored and how long can it be kept?
7. What cannot be sterilized in a hot air sterilizing oven.
8. List the steps in proper glassware washing.

Topic: Sampling

1. What chemical is used to dechlorinate a sample?
2. What happens to the bacterial population if the sample is not chilled?
3. Why is a string or slip of paper put between the sample bottle mouth and the ground glass closure before sterilizing?

Topic: Dilution

1. How many mls. of sterile distilled buffered water should be in the dilution blank?
2. Diagram how to get a 1:10000 dilution.

Topic: Membrane Filtration Procedure

1. Why is the work area disinfected immediately before testing begins?
2. Why is the filtering funnel rinsed with sterile distilled buffered water after the sample is filtered?
3. What traps the bacteria and provides a surface for colony growth when a sample is filtered.
4. How are sterile membrane filters handled?

5. Why is the culture dish inverted during incubation?

6. What is the proper incubation time and temperature for the fecal coliform test?

Topic: Counting Procedure and Data Interpretation and Evaluation

1. What is the proper counting range?

2. Describe the appearance of a fecal coliform colony when grown on m-FC media.

3. Give the formula for computing the number of fecal coliform bacteria per 100 mls. sample.

Module No:	EVALUATION - PART B

Instructor Notes:	Instructor Outline:

Answers

Topic: Introduction

1. Insufficient treatment or no chlorination.

2. The cold temperature will aide in stream reclaimation by rapidly killing the bacteria.

3. (a) Rod shaped
   (b) Negative
   (c) Fecal matter
   (d) 44.5

4. 400 per 100 mls.

Topic: Laboratory Equipment

1. Sterilizing heat stable equipment and liquids.

2. A circulated water bath.

3. Vacuum source
   Vacuum tubing
   Filtering flask
   Filtering funnel
   Membrane filter

4. (1) To protect the sample from contamination.
   (2) To protect the lab technician from contamination.

5. Rinsing removes detergent residue which can inhibit bacterial growth.

6. Sterilizing kills the bacteria thereby protecting the environment when the cultures are disposed of.

Module No:	EVALUATION - PART B

Instructor Notes:	Instructor Outline:

Answers

Topic: Sampling

1. Sodium Thiosulfate
2. It will change, first with growth followed by rapid die off.
3. To keep them from sticking together due to a vacuum being formed in the bottle while cooling.

Topic: Dilution

1. 99 mls. ± 2.0 mls.
2. Sample 1 ml. → 99 ml. dilution blank
   ↓ 1 ml.
   This is the 1:10000 dilution → 99 ml. dilution blank

Topic: Membrane Filtration Procedure

1. Disinfection removes most dust and bacteria from the work area and this lowers the risk of contamination.
2. The rinses remove the bacteria which adhered to the sides of the funnel and deposits them on the membrane filter.
3. The membrane filter
4. Membrane filters are handled by flamed forceps on the outer 1/8 inch only.

Module No:	EVALUATION - PART B

Instructor Notes:	Instructor Outline:

Topic: Laboratory & Media Preparation

Answers

1. (1) General laboratory cleanup
   (2) Cleaning up spilled bacterial cultures

2. It allows the equipment to be stored without becoming contaminated.

3. (1) Purchase a distillation unit and make it.
   (2) Purchase the distilled water from a reliable source.

4. (1) Peptone
   (2) KH_2PO_4 (Potassium dihydrogen phosphate)

5. m-FC growth media is not sterilized because it contains heat sensitive components which will be destroyed at sterilizing temperatures.

6. Keep the 1% Rosalic Acid solution in the refrigerator for no more than 1 month.

7. Rubber, plastic and paper items and all liquids.

8. (1) Wash in hot soapy water
   (2) Rinse in hot tap water 6 - 12 times
   (3) Rinse 1 - 3 times with distilled water
   (4) Air dry
   (5) If spots appear when dry, rewash.

Module No:	EVALUATION - PART B

Instructor Notes:	Instructor Outline:

Answers

5. To keep the moisture under the membrane - if moisture collects on the lid, it will drip onto the membrane surface and distort the colony growth.

6. 44.5° C. ± 0.2° C. for 24 ± hrs.

Topic: Counting Procedure and Data Interpretation and Evaluation

1. 20 - 60 fecal coliform colonies

2. Dark blue bacterial colony.

3. $\frac{\text{count}}{\text{amount filtered (mls.)}} \times 100$